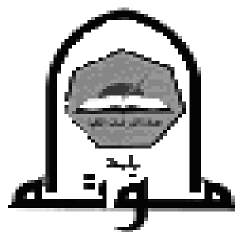


Mu'tah University
Deanship of Graduate studies

Effects of some heavy metals on the activity and kinetics of Peroxidase and Polyphenol oxidase enzymes in *Petroselium cripsum, Rosmarinus officinalis and Eruca Sativa*

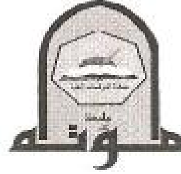
By
Manar Abdul Salam Al-Btoush

Supervisor
Dr. Omar Atrooz

A Thesis Submitted to the Deanship of the Graduate studies in Partial fulfillment of the Requirements for the Degree of Master in Biology – Department of biology

Mu'tah University, 2013

الآراء الواردة في الرسالة الجامعية لا تُعبر
بالضرورة عن وجهة نظر جامعة مؤتة



MUTAH UNIVERSITY
Deanship of Graduate Studies

جامعة مؤتة
عمادة الدراسات العليا

نموذج رقم (14)

قرار إجازة رسالة جامعية

تقرر إجازة الرسالة المقدمة من الطالبة منار عبدالسلام البطوش الموسومة بـــ:

Effects of Some heavy metals on the activity and kinetics of Peroxidase and Polyphenol oxidase enzymes in *Petroselium cripsum, Rosmarinus officinalis and Eruca Sativa*

استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم الحياتية.

القسم: العلوم الحياتية.

	التوقيع	التاريخ	
د. عمر محمد عطروز		30/12/2013	مشرفاً ورئيسا
أ.د. جمعة مطيع الشخانبة		30/12/2013	عضواً
أ.د. خالد محمد خليفات		30/12/2013	عضواً
د. سميح عبدالكريم الصريرة		30/12/2013	عضواً

عميد الدراسات العليا

د. علي الضمور



MUTAH-KARAK-JORDAN
Postal Code: 61710
TEL :03/2372380-99
Ext. 5328-5330
FAX:03/ 2375694
e-mail: dgs@mutah.edu.jo sedgs@mutah.edu.jo
http://www.mutah.edu.jo/gradest/derasat.htm

مؤته – الكرك – الاردن
الرمز البريدي :61710
تلفون :03/2372380-99
فرعي 5328-5330
فاكس 03/2 375694
البريد الالكتروني
الصفحة الالكترونية

Dedication

This thesis is dedicated to my parents. specially to my mom my best friend for her love and support.

To my brothers and sisters, and to every one who have always helped me and believed that I could do it.

Manar A.Al-Btoush

Acknowledgment

The most thanks to ALLAH who guided me during my life and is still supporting me with his mercy and giving.

I would like to thank my parents for their endless love and supporting me spiritually throughout my life.

I would like to express my sincere gratitude to my advisor Dr. Omar Atrooz for his guidance helped me in all the time of research and writing up of this thesis.

Besides my advisor, I would like to thank the rest of my thesis committee: Prof. Khaled Khliefat, Prof. Jumah Shakanbeh, and Dr. Sameeh Sarayrah, for their encouragement and insightful comments.

I would like to express my deepest appreciation to all those who provided me the possibility to complete this thesis, especially my childhood friend, my brother Dr. Mohammad Hujouj.

I cannot find words to express my gratitude to chairman of biology department Dr. Muhammad Al- limuon for his supporting.

I am indebted to my colleagues in biology department who supported me and to Mr. Mohammad Thonebat who designed my thesis.

Manar A. Al-Btoush

Table of Contents

List of Tables

List of Figures

List of Appendixes

List of Abbreviations

RNS	Reactive nitrogen species
ROS	Reactive oxygen species
HM	Heavy metal
$O_2^{\cdot -}$	superoxide radicals
$^1O_2$	Singlet oxygen
$OH^{\cdot}$	Hydroxyl radical
ATP	Adenosine triphosphate
HO	Hydroxyl radical
$O^{\cdot}_2$-	Superoxide radical
H_2O_2	Hydrogen peroxide
EIS	Enzyme- Inhibitor- Substrate complex.
K_m	Michaelis's constant
V_{max}	Maximum velocity
K_i	An equilibrium constant for the inhibitor.
PPO	Polyphenol oxidase
POX	Peroxidase
CAT	Catalase
SOD	superoxide dismutase
PCD	programmed cell death
WHO	World Health Organization
NCI	Noncompetitive inhibitor
BSA	Bovine Serum Albumin
L-DOPA	L-3,4-dihydroxyphenylalanine

Abstract

Effects of some heavy metals on the activity and kinetics of Peroxidase and Polyphenol oxidase enzymes in *Petroselium cripsum, Rosmarinus officinalis* and *Eruca Sativa*

Manar A.Al-Btoush

Mu'tah University, 2013

The catalytic activities and the kinetic parameters of crude enzymes polyphenol oxidase (POX) and peroxidase (PPO) ezymes of crude extracts of *Rosmarinus officinalis, Petroselium cripsum*, and *Eruca Sativa* were invistigated in the presence and absence of various heavy metals.

The results showed that the crude enzyme (PPO) activity in the selected plants were increased in the presence of 400 µM of Cd^{2+}, Co^{2+}, Al^{3+}, Fe^{3+} and Ni^{2+} as evidenced by the decrease in the K_m and the increase in the V_{max} values. Meanwhile, the presence of some other heavy metals resulted in the activation of crude enzyme (PPO) extracts of *Eruca Sativa* and *Rosmarinus officinalis* such as Mo^{2+}. In contrast, other heavy metals such as $Hg^{2+}$and Cr^{2+}acted as a noncompetitive inhibitors in the crude enzyme (PPO) extracts of *Petroselium cripsum*, while Fe^{3+} and Pb^{2+} in crude enzyme (POX) of *Rosmarinus officinalis* and *Eruca Sativa* and Cr^{+2} and Co^{2+} in the *Eruca Sativa* extract (POX). Moreover, Al^{3+} and Mo^{2+} acted as a noncompetitive inhibitors in the crude enzyme (POX) *Rosmarinus officinalis* extract.

Uncompetitive inhibition were observed in presence of Cu^{2+} and Pb^{2+} for crude enzyme (PPO) extract of rosemary as evidenced by the decrease in the K_m and the V_{max} values. In presence of Zn^{2+}, the results revealed that the activity of crude enzyme (PPO) of *Petroselium cripsum* were increased (activation). Meanwhile, the presence of Zn^{2+} in the crude extract of *Rosmarinus officinalis* cause a noncompetitive inhibition and uncompetitive inhibition for PPO extracted in *Eruca Sativa*.

Regarding the effects of heavy metals on peroxidase activity, presence of Co^{2+}, Fe^{3+}, Hg^{2+} and Zn^{2+}in the crude enzyme (POX) extract of *Petroselium cripsum* and *Eruca Sativa* have an activation effects . In contrast, Cd^{2+}, $Co^{2+}$and Cu^{2+} have an uncompetitive inhibition for the selected plants.

Furthermore, Fe^{3+}, Al^{3+}, Pb^{2+} and Mo^{2+} were found to act as a noncompetitive inhibitors on the crude enzyme (POX) extract of *Rosmarinus officinalis*, while Cr^{+2}, Co^{2+}, Fe^{3+} and Pb^{2+} acted as a noncompetitive inhibitors on the crude enzyme (POX) extract *Rosmarinus officinalis*.

Presence of heavy metals altred these activities by acting as a uncompetitive or noncompetitive inhibitors or acting as activators depending on the type of heavy metals.

الملخص

تاثير بعض المعادن الثقيلة على نشاط إنزيمات البيروكسيديز و البولي فينول أوكسيديز في البقدونس، إكليل الجبل والجرجير

منار عبدالسلام البطوش

جامعة مؤتة، 2013م

تم إستخلاص إنزيمات البولي فينول أوكسيديز(PPO) والبيروكسيديز (POX) الخام من اوراق كل من إكليل الجبل والبقدونس والجرجير. لقياس النشاط التحفيزي للانزيمات الخام في حضور وغياب عدد من المعادن الثقيلة. أظهرت النتائج أن نشاط الانزيم الخام البوليفينول أوكسيديز في النباتات الثلاثة المختارة ازداد تحت تأثير 400 ميكرومولار من Fe^{3+}, Al^{3+}, Co^{2+} , Cd^{2+} و Ni^{2+} بدلالة الانخفاض في قيم (K_m) والزيادة في قيم (V_{max}). بينما وجود Mo^{2+} يزيد نشاط الانزيم (PPO) فقط في البقدونس و الجرجير، ولكنه يعتبر مثبط غير تنافسي (noncompetitive inhibition) لنشاط PPO في إكليل الجبل. وجود Hg^{2+} و Cr^{+2} يزيد نشاط PPO في كل من الجرجير و إكليل الجبل، بينما في البقدونس يؤثران كمثبط غير تنافسي.

وقد لوحظ تثبيط لاتنافسي (Uncompetitive inhibition) في وجود Cu^{2+} و Pb^{2+} من إكليل الجبل بدلالة الانخفاض في قيم (K_m) و (V_{max}). من ناحية أخرى، كلا المعدنين تسببت في زيادة النشاطية ل PPO في الجرجير والبقدونس . في وجود Zn^{2+} أظهرت النتائج أن نشاط PPO في البقدونس قد زاد. وفي الوقت نفسه ، فإن وجود Zn^{+2} أدى الى تثبيط غير تنافسيي ل PPO المستخرج من إكليل الجبل وتثبيط لاتنافسي (Uncompetitive inhibition) ل PPO المستخرج من الجرجير.

فيما يتعلق بتأثير المعادن الثقيلة على نشاط البيروكسيديز (POX) ، أظهرت النتائج ازدياد في نشاط الانزيم الخام في إكليل الجبل في وجود 400 ميكرومولار من Cr^{2+} بدلالة الانخفاض في قيم (K_m) والزيادة في قيم (V_{max}) . وفي الوقت نفسه، زاد النشاط البيروكسيديز من البقدونس و الجرجير في وجود Hg^{2+},Fe^{3+}, Co^{2+} و Zn^{2+} ، على التوالي.

وقد لوحظ تثبيط لاتنافسي (Uncompetitive inhibition) في وجود Cu^{2+} Ni^{2+}, Cd^{+2} ل البيروكسيديز من النباتات الثلاثة. كما لوحظت تثبيط لاتنافسي في النشاط أيضا في وجود AL^{3+} و Mo^{2+} ل انزيم البيروكسيديز من البقدونس و الجرجير. كما تسبب Zn^{2+} بتثبيط لاتنافسي لنشاط البيروكسيديز المستخرج من البقدونس وإكليل الجبل . وقد انخفض نشاط البيروكسيديز من إكليل الجبل (تثبيط غير تنافسي) في حضور Hg^{2+}, Co^{2+} . في حين أن نشاط البيروكسيديز من البقدونس تناقص في حضور Pb^{2+} و Cr^{2+} . ومع ذلك ، فقد لوحظ تثبيط غير تنافسية في وجود Fe^{3+} و Pb^{2+} في كل من إكليل الجبل والجرجير. كما لوحظ أن نشاط البيروكسيديز من الجرجير انخفض (تثبيط غير تنافسية) في حضور Cr^{2+} و Co^{2+}. في المقابل فإن Al^{3+} و Mo^{2+} تسبب في تثبيط غير تنافسي في نشاط البيروكسيديز المستخرج من إكليل الجبل.

Chapter One
Theoretical Background

1.1 Introduction:

Oxygen plays a major role in energy metabolism as the final acceptor for electrons in the electron transport chain. However, when cells use oxygen to produce energy, free radicals are created as a consequence of ATP realization by the mitochondria. These by-products are mostly reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) generated from various cellular redox processes (Halliwell *et al.*, 2007). These reactive species play a dual role as both toxic and beneficial compounds. At low levels, ROS and RNS exert helpful effects on cellular responses and immune function (Young and Woodside, 2001). On the other hand, at high concentration, free radicals and other oxidant agents produce oxidative stress that can damage cell structures such as lipids, proteins, and DNA (Valko *et al.*, 2005).

In general, free radicals can be categorized as endogenous and exogenous free radicals. Endogenous free radicals are those generated as a consequence of immune cell activation, inflammation, mental stress, immoderate exercise, ischemia, infection, cancer and aging. While, exogenous ROS and RNS are those sourced from polluted air and water, smoking, alcohol, heavy or transition metals, certain drugs, pesticides and industrial solvents, cooking (smoked meat, used oil, fat), radiation (increased exposure to sunlight), etc. (Young and Woodside, 2001; Valko *et al.*, 2005; Chatterjee *et al.*, 2007).

Particularly, heavy metals are defined as that group of elements that have specific weights higher than about $5g/cm^3$. A number of them such as cobalt, iron, manganese, molybdenum, nickel, zinc and copper are essential micronutrients for normal biochemical and physiological functions such as redox reactions, electron transfer and other important metabolic processes in plants. Other heavy metals which are nonessential (Pb^{+2}, Cd^{+2}, Cr^{+2}, Hg^{+2} etc.) are potentially highly toxic for plants (Sebastianil *et al.*, 2004; Rai *et al*., 2004). Recently, the increasing accumulation of these toxic heavy metals in large areas of lands resulting from urban activities was stressed. Their impacts on the plantation and the quality of the produced crops were also studied (Khan *et al*, 2000; Clemens, 2001).

The excessive concentrations of certain trace elements such as Cd^{+2}, Co^{+2}, Cr^{+2}, Hg^{+2}, Mn^{+2}, Ni^{+2}, Pb^{+2} and Zn^{+2} usually toxic and lead to growth inhibition and decrease in biomass and consequentially the death of the plant (Zenk, 1996). It has been reported that the contamination with heavy metals inhibit certain physiological processes such as respiration, photosynthesis, cell elongation, plant-water relationship, N-metabolism and mineral nutrition (Zornoza, 2002).

Plants under stress exert some defense mechanisms to protect themselves from the harmful effect of oxidative stress. ROS scavenging is one among the common defense reaction against abiotic stresses (Vranova *et al.*, 2002; Jaleel *et al.*, 2007b). ROS scavenging depends on the detoxification mechanism provided by an integrated system of non-enzymatic reduced molecules (antioxidants) and enzymatic antioxidants such as polyphenol oxidase and peroxidase (Jaleel *et al*., 2007c).

The roles of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects (Willcox *et al*., 2004; Lien Aipham-Huy *et al*., 2008) in one of the following two ways:

1. Chain-breaking, when a radical releases or steals an electron, a second radical is formed, The last one do the same action on another molecule and continues until either the free radical formed is stabilized by a chain-breaking antioxidant (vitamin C, E, carotenoids, etc) or it simply settle into an safe product.
2. The preventive way, an antioxidant enzyme like superoxide dismutase, catalase and glutathione peroxidase can prevent oxidation by lowering the rate of chain initiation, e.g., either by scavenging initiating free radicals or by stabilizing transition metal radicals such as copper and iron (Young and Woodside, 2001).

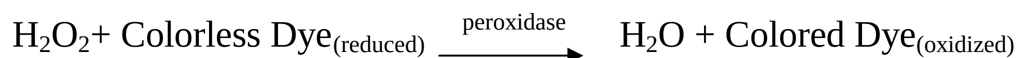
Polyphenol oxidase (PPO):

Previously, it has been reported that, about 50% of tropical fruits were rejected due to quality defects resulting from enzymatic browning (Whitaker, 1996). The browning of the fruits is mainly catalyzed by the enzyme polyphenol oxidase (1, 2 benzenedio; oxygen oxidoreductase; EC 1.10.3.1) which is also known as phenoloxidase, phenolase, monophenol oxidase, diphenol oxidase and tyrosinase (Marshall *et al*., 2000).

PPO is a copper associated enzyme with two binding sites for phenolic substrates. It is located in the chloroplast bound to thylakoid membranes. PPO is activated by releasing into the cytosol when plant tissues undergo physical damage such as bruising, cutting or blending.

Peroxidase (POX):

Peroxidases are group of enzymes found in animal and plant tissues as well as in microorganisms that catalyze the oxido-reduction reaction between H_2O_2 and various reductants.



$$H_2O_2 + \text{Colorless Dye}_{(reduced)} \xrightarrow{\text{peroxidase}} H_2O + \text{Colored Dye}_{(oxidized)}$$

Besides their main function as a catalysts in the oxido-reduction reaction between H_2O_2 and various reductants, peroxidase (POX) in plants also participates in many other functions such as hormone regulation, defence mechanisms, indolacetic degradation and lignin biosynthesis (Serrano *et al*., 2008).

Eruca sativa **L**. a member of the Brassicacae family, has gained greater importance as a salad vegetable and spice (Schröder *et al*., 2008). It has various medicinal and therapeutic properties including inhibition of tumorigenesis (Lynn *et al*., 2006) anti-ulcer and hepatoprotective activities(Alasoumi *et al*., 2008). Rocket, locally known as Jarjeer.

Rosmarinus officinalis **L**. belong to family Lamiacea. Rosemary is a common household aromatic plant used for flavoring food and cosmetics. In folk medicine it is used as an antispasmodic in renal colic and dysmenorrhoea, in relieving respiratory disorders and to stimulate growth of hair. Extract of rosemary relaxes smooth muscles of trachea and intestine, and has choleretic, hepatoprotective and antitumerogenic activity (Al-Sereitia *et al*., 1999).

Petroselinum crispum is an important culinary herb native to the Mediterranean area. Parsley is a member of the Umbelliferae family that has been employed in the food, pharmaceutical, perfume, and cosmetics industries (Lopez *et al*., 1999). In folk medicine used to treat a wide variety of diseases (Ozsoy-Sacan *et al*., 2006).

However, besides their use in folk medicine, as food flavoring, cosmetics and pharmaceutical products *Petroselium cripsum, Rosmarinus officinalis* and *Eruca Sativa* as many other herbs and spices contain antioxidant effective components and antioxidant enzymes that retards oxidation (Sasse *et al.*, 2009) and can be affected by heavy metals contamination in soil and water which lead to the deterioration of the quality of the crops.

Therefore, the recent study aims:

1. To determine the enzymatic activities and kinetics of the antioxidant enzymes peroxidase and polyphenoloxidase in *Petroselium cripsum, Rosmarinus officinalis* and *Eruca Sativa* plants.

2. To study the effects of selected heavy metals on the activity and kinetics of the above antioxidant enzymes in *Petroselium cripsum, Rosmarinus officinalis* and *Eruca Sativa* plants.

Chapter Two
Literature Review

2.1 Antioxidants:

An antioxidant is a substance that retards oxidation by repressing free radical initiation or producing more free radicals which can resume the reaction (Fennema, 1996). Antioxidants can bind metals, scavenge species that perpetuate oxidation, quench high-energy oxygen species-preventing formation of peroxides, or decompose lipid peroxides. They can get better color and flavor stability in meats (Xiong *et al*., 1993). Some vitamins (ascorbic acid, vitamin E) manifest antioxidative activity. Many herbs and spices (rosemary, oregano, grapeseed, sage, thyme) contain antioxidant effective components that is due primarily to phenolic -OH groups (Cai *et al*., 2005; Rojas and Brewer, 2007; Sasse *et al*, 2009).

2.1.1 Reactive oxygen species (ROS):

Reactive oxygen species (ROS) are continuously produced as a by-products of various metabolic pathways that are localized in different cellular compartments such as chloroplast, mitochondria and peroxisomes (Halliwell, 2006; Del Rio *et al.*, 2003). Figure (1) shows different sites of production of reactive oxygen species (ROS) in plant cells. The ROS comprises both free radical (superoxide radicals, hydroxyl radical, perhydroxy radical and alkoxy radicals) and non-radical (molecular) forms (H_2O_2, hydrogen peroxide and singlet oxygen) (Gill and Tuteja, 2010). However, beside their normal production as end product of oxidative metabolism various abiotic stresses lead to the over production of ROS in plants which are highly reactive and toxic and cause damage to proteins, lipids, carbohydrates and DNA which ultimately results in oxidative stress (Foyer and Noctor, 2005).

In chloroplasts, photosystem I and II (PSI and PSII) are the major sites for the production of $^1O_2$ and O_2 (Hatz, 2007). The water-water cycle channels electrons obtained from the splitting of water molecules at PSII through the photosynthetic apparatus. These electrons are transferred to oxygen by PSI and result in the formation of superoxide radicals.

A membrane-attached copper/zinc superoxide dismutase converts the superoxide radicals into hydrogen peroxide, and a membrane-bound ascorbate peroxidase (thylakoid-APX) converts the hydrogen peroxide back into water (Heber, 2001). In mitochondria, complex I, ubiquinone and complex III of electron transport chain (ETC) are the major sites for the generation of O_2 (Moller, 2001; Sweetlove and Foyer, 2004) .

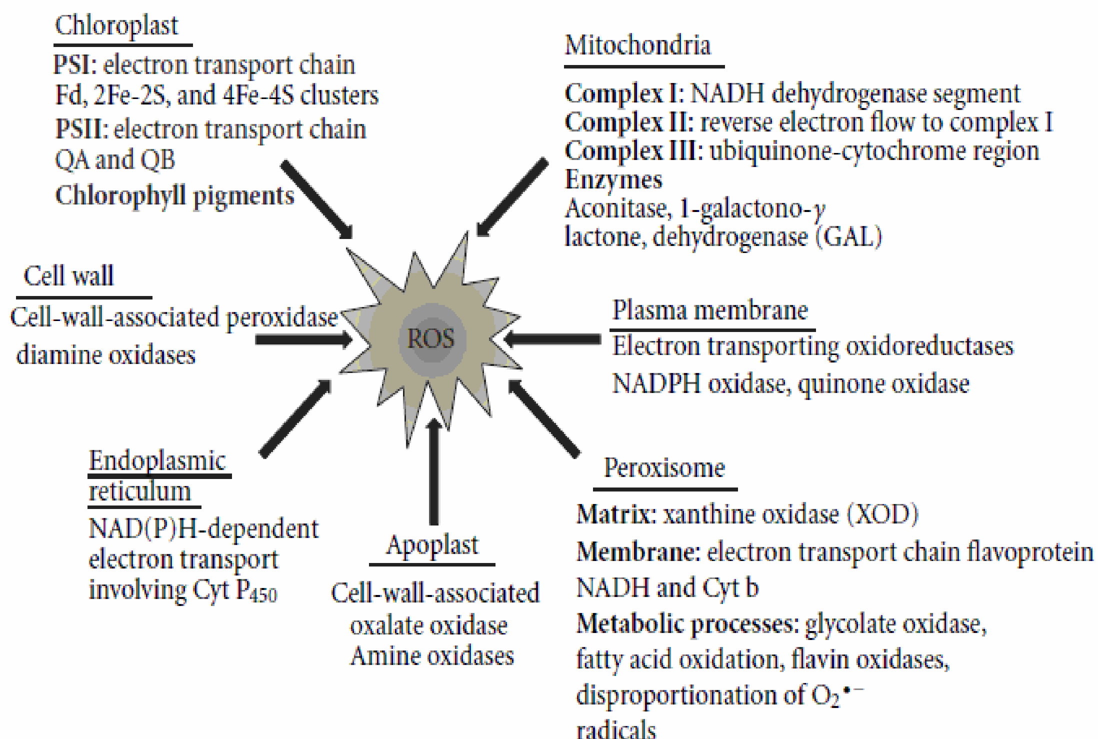


Figure (1)
Sites of production of reactive oxygen species (ROS) in plants
(Hasanuzzaman, *et. al.*, 2013)

ROS also induce the expression of a number of genes and therefore control many processes like growth, cell cycle, programmed cell death (PCD), abiotic stress responses, pathogen defense, systemic signaling and development (Dalton *et al*., 1999; Sharma and Dubey, 2005).

H_2O_2 plays a dual role in plants: at low concentrations, it acts as a signal molecule involved in acclimatory signaling triggering tolerance to various biotic and abiotic stresses (Laloi *et al*., 2004; Fukao and Bailey-Serres 2004; Mittler *et al*., 2004) and at high concentrations, it leads to PCD (Dat *et al*. 2000). H_2O_2 has also been shown to act as a key regulator in a broad range of physiological processes, such as senescence (Bhattacharjee 2005), photorespiration and photosynthesis (Rasmusson *et al*., 1998), stomatal movement (Pei *et al*., 2000; Neill *et al*., 2002), cell cycle (Suzuki *et al*., 1999) as well as growth and development (Papadakis and Roubelasis-Angelakis, 2002). H_2O_2 is starting to be accepted as a second messenger for signals generated by means of ROS because of its relatively long life and high permeability across membranes (Desikan *et al*., 2003).

2.1.2 Antioxidant importance:

Plants have very efficient enzymatic (superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, monodehydroascorbate reductase, dehydroascorbate reductase, glutathione peroxidase, guaicol

peroxidase and glutathione-S- transferase) and non-enzymatic (ascorbic acid, glutathione, phenolic compounds, alkaloids, non-protein amino acids and a-tocopherols) antioxidant defense systems which work in harmony to control the cascades of uncontrolled oxidation and protect plant cells from oxidative destruction by scavenging of ROS (Mittler *et al*., 2004). In addition to the efficient enzymatic antioxidant defense system, plant cells contain chelating agents that bind metal ions (iron, copper) to prevent them from participation in oxidation reactions (Fennema, 1996).

Antioxidants usually contain aromatic rings that donate $H^{\bullet}$ to the free radical formed during lipid oxidation (Figure 2). They "sacrifice themselves" by giving up a hydrogen atom, then rearrange to a stable conformation. High levels of phenolic compounds found in Plant extracts (clove,cinnamon, marjoram, oregano, cumin, rosemary) have strong $H^{\bullet}$ donating activity and effectively scavenge H_2O_2 and ROS (Lugasi *et al*., 1995). The free-radical-scavenging potential of natural polyphenolic compounds depends on the number and arrangement of free –OH groups on the flavonoid skeleton (Lupea *et al*., 2008; Shahidi and Wanasundara, 1992; Kondo *et al*., 2001). Flavonoids can lose a hydrogen-reducing metal rendering them less pro-oxidative (Fernandez *et al*, 2002).

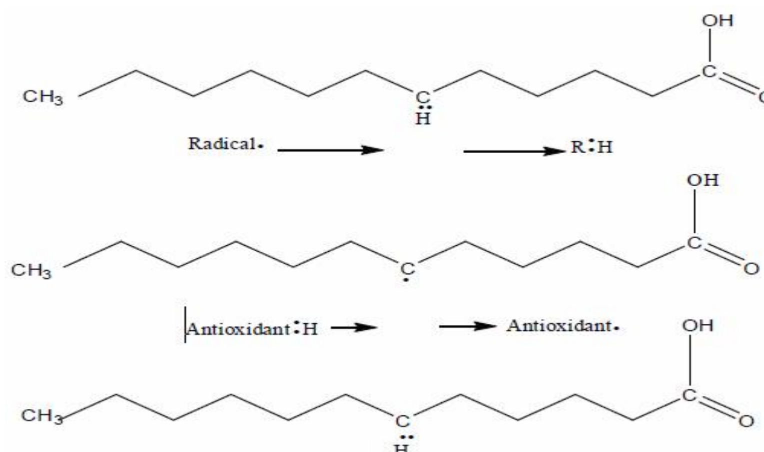


Figure (2)
Antioxidant interaction with fatty acid free radical
(Lugasi *et al*., 1995).

2.1.2 Antioxidant enzymes.

2.1.2.1 Peroxidase (POX) (EC 1.11.1.7)

H_2O_2 is a common end product of oxidative metabolism, and becomes a strong oxidizing agent, which harmful to the cell if allowed to accumulate. So, peroxidases eliminate excess electron acceptor compounds such as superoxide radicals, hydrogen peroxide and lipid peroxidase from plant cells under normal and stress conditions (Laloue *et al*., 1997). POX also involved in enzymatic browning since diphenols may function as

reducing substrate in this reaction (Figure 3) (Chisari *et al*., 2007).

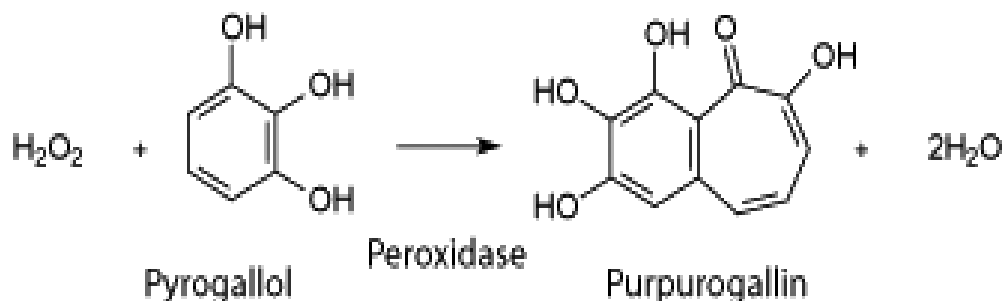


Figure (3)
POX reducing reaction
(Chisari *et al*., 2007)

2.1.2.2 Polyphenol oxidase (PPO) (E.C. 1.14.18.1)

The catalytic action of polyphenol oxidase is related to undesirable browning and off-flavor generated in stored and processed foods. Because the phenomena decreases fruit quality, PPO has been regarded to be a critical enzyme in food technology (Joo Young Kim *et al*., 2001). On the other hand, PPO has been also shown to have important applications such as using in the synthesis of useful added products like the substituted catechol, L-DOPA for the treatment of Parkinson's disease (Pialis and Saville, 1998).

A number of other catechols have applications as fine chemicals or as starting materials for pharmaceutical drug synthesis (Halder *et al.*, 1998). PPOs can be considernas efficient reagents for cleaning polyphenols-containing wastewater (Freire *et al.*, 2002).

Polyphenol oxidase catalyzes two types of the oxidative reaction involving molecular oxygen: the hydroxylation of monophenols to

o-diphenols and the oxidation of o-diphenols to o-quinones (Figure 4), which lead to the formation of black or brown pigments (Lee *et al.*, 2007).

Quinones formed during PPO oxidation reaction may undergo redox recycling, which generates radicals and can damage DNA and proteins (Hill, 1992; Yoruk and Marshall, 2003).

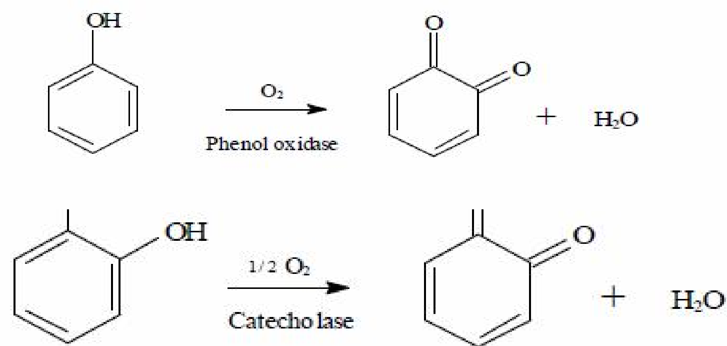


Figure (4)
PPO oxidation reaction
(Lee *et al.*, 2007).

2.2 Heavy metals stress:

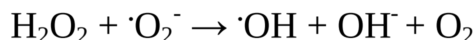
Heavy metal (HM) affects plants in two ways. Firstly, it alters reaction rates and impacts the kinetic properties of enzymes which cause changes in plant metabolism. Secondly, excessive heavy metals lead to oxidant stress (Schützendübel and Polle, 2002). HM toxicity is one of the major abiotic stresses leading to excessive accumulation of ROS and methylglyoxal (MG), which can cause peroxidation of lipids, oxidation of protein, inactivation of enzymes, DNA damage and/or interact with other vital components of plant cells. Recently, Al, Zn, Ca, Mg and Cd have been found to induce phenoxyl radical-induced lipid peroxidation (Sakihama *et al.*, 2002).

Plant cells have evolved several defence mechanisms to control the damages caused by ROS. Primary prevent metal to enter into the cell via exclusion, or binding of metal to cell wall and other ligands, amino acids, glutathione or phytochelatins to become harmless (Antosiewicz and Wierzbicka, 1999). Secondary defence system includes various antioxidants and enzymatic mechanisms to resist an increased production of ROS caused by the metal (Mittler, 2002). If the local antioxidant cannot overcome ROS production, ROS diffuse to the cytosol and other compartments (figure 5).

Heavy metals induce oxidative stress in cells and tissues in the following ways:

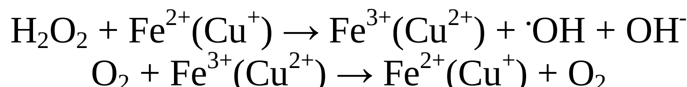
1. Generation of ROS, H_2O_2 in the presence of $^{\cdot}O_2^-$ can generate highly reactive $^{\cdot}OH$ *hydroxyl radicals* via the metal-catalyzed Haber-Wiess reaction thus the scavenging of H_2O_2 in cells is critical to avoid oxidative damage (Inze and Van, 1995). In the presence of redox active transition metals such as Cu^+ and Fe^{2+}, H_2O_2 can be converted to $^{\cdot}OH$ molecule in a metal-catalyzed reaction via the Fenton reaction (Mtthofer *et al.*, 2004; Wo, 1997).

Haber-Wiess reaction



$$H_2O_2 + {}^{\cdot}O_2^{-} \rightarrow {}^{\cdot}OH + OH^{-} + O_2$$

Fenton reaction



$$H_2O_2 + Fe^{2+}(Cu^{+}) \rightarrow Fe^{3+}(Cu^{2+}) + {}^{\cdot}OH + OH^{-}$$
$$O_2 + Fe^{3+}(Cu^{2+}) \rightarrow Fe^{2+}(Cu^{+}) + O_2$$

2. Blocking of essential functional groups in biomolecules: proteins (by the inactivation of the SH-groups in enzymes active centers) and polynucleotides (Baranowska –morek, 2003; Mtthofer *et al.*, 2004). H M mainly inactivate the antioxidant enzymes responsible for free radical detoxification.
3. Substitution of essential metal ions by other incorrect ones (Rai *et al.*, 2004). Numerous enzymes need cofactors to work properly for both HM ions (such as Fe^{2+}, Mg^{2+}, Cu^{2+}, Ca^{2+}) and organic molecules (such as haem, biotin, FAD, NAD, or coenzyme A). If the essential metal ions from specific binding sites displaced, the enzyme will lose its function (Sharma and Dietz, 2009; Sch¨utzend¨ubel and Polle, 2002). Divalent cations such as Co^{2+}, Ni^{2+}, and Zn^{2+} displace Mg^{2+} in ribulose-1,5- bisphosphate-carboxylase/oxygenase (RuBisCO) and result in a loss of activity (Van Assche and Clijsters, 1986). Displacement of Ca^{2+} by Cd^{2+} in calmodulin, an important protein in cellular signaling, led to the inhibition of calmodulin-dependent phosphodiesterase activity in radish (Rivetta *et al.*, 1997).

However, HM can also cause membrane damage through various mechanisms, including the oxidation of and cross-linking with protein thiols, inhibition of key membrane protein such as H^{+}-ATPase, or causing changes in the composition and fluidity of membrane lipids (Meharg, 1993).

In addition to their harmful effects on cells, ROS (especially H_2O_2) act as a key molecule that lead stimulate signal transduction and HM tolerance in plants (Seth *et al.*, 2012**)** in stress response and modulate the activation of stress responsive pathways, proteins, and genes (Sharma and. Dietz, 2009;; Hossain *et al*, 20011; Mittler *et al,* 2004).

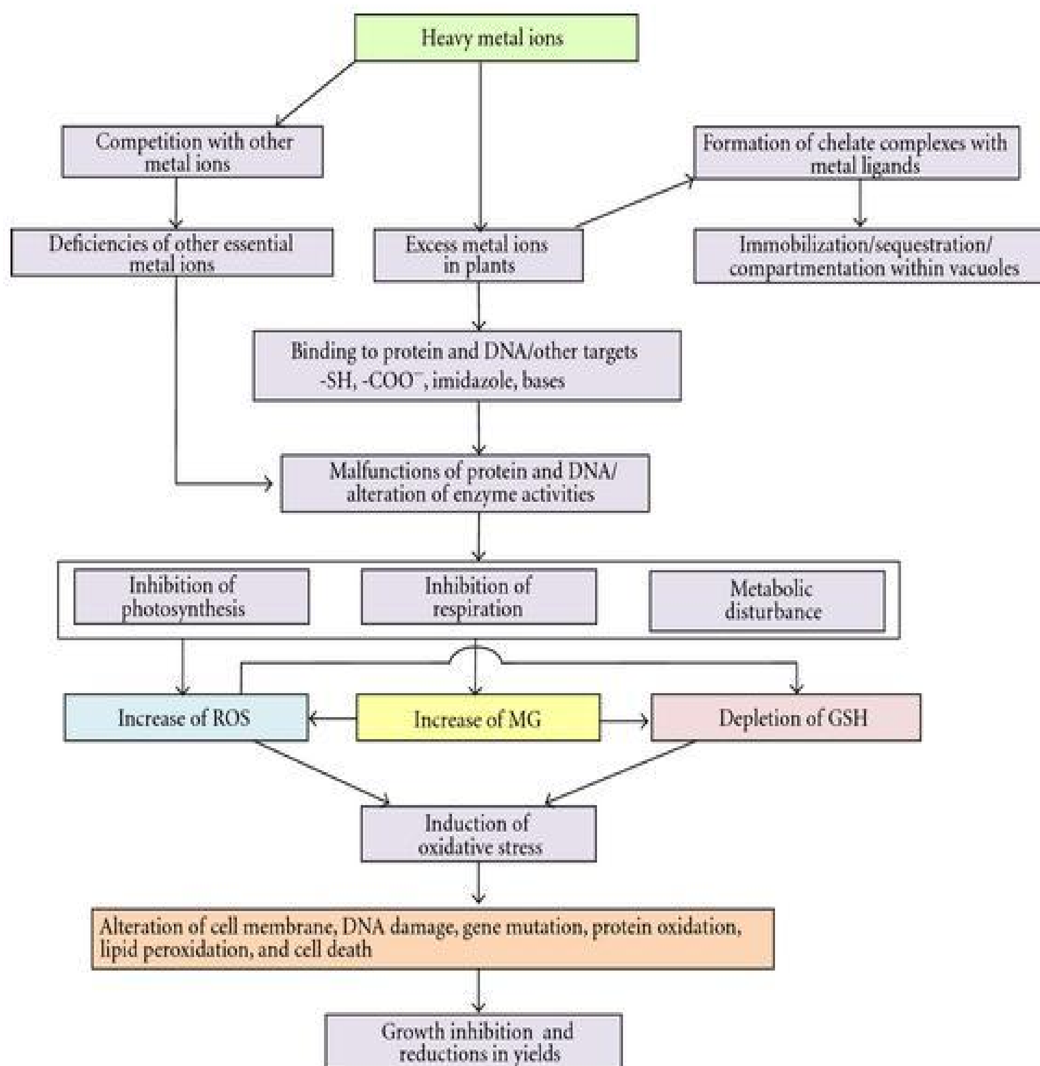


Figure (5)
Summary of Possible biochemical and molecular mechanisms of heavy metal-mediated ROS induction and damage to the development of higher plants
(Hossain *et al.*, 2012).

2.3.1 Lead (Pb)

Lead (Pb^{2+}) is one of the most toxic HM and considered as serious environmental pollutant with specific toxic actions. Pb^{2+} inhibits metabolic processes such as nitrogen assimilation, photosynthesis, respiration, water uptake and transcription (Kupper *et al*., 2007; John *et al*., 2008). Binding of nucleic acids to Pb^{2+} causes aggregation of chromatin, and inhibit replication and transcription (De Vos *et al*., 1992). Pb^{2+} causes harmful effects in biological systems by inactivating several enzymes by binding with their SH-groups (Rauser, 1991). Pb^{2+}decreases the efficiency of oxidation-reduction enzymes or the electron transport system leading to fast production of ROS in the cell (Stroinski and Kozlowska, 1997). In addition, Pb^{2+} affects membrane-related functions such as the activity of

membrane enzymes, endo- and exocytosis, transport of solutes across the bilayer, and signal transduction processes by causing lateral phase separation (Adonaylo and Oteiza, 1999).

2.3.2 Molybdenum (Mo)

Molybdenum is an essential nutritional element required for the normal growth of most of the plants. It diffuses to the soil by using industrial stainless steel, mining, cast iron, and agricultural activities (Mendel, 2005). Mo increases soil pH which consequently increases their uptake by the plants (Smith, 1997). The absorption of excess Mo may result in toxicity symptoms, induce antioxidant activity and accumulation of anthocyanin in plant tissues to cope the excess metal stress (Stroud, 2010).

2.3.3 Iron (Fe)

Iron is an essential element in many physiological functions, and its homeostasis is strictly regulated by various mechanisms. In biological systems iron exists in three oxidation states (II, III, and IV). The majority of iron in the organism is bound to haemoglobin, transferrin, ferritin, and iron-containing enzymes. Therefore, only a trace amount of free iron is present (Valko *et al*., 2005).

Iron is an essential element for photosynthesis, respiration, nitrogen fixation, DNA and hormone synthesis in plants (Briat and Lobre´aux 1997; Becana *et al*., 1998; Schmidt 2003). Iron is a major constituent of important proteins (ferredoxin and cytochromes) and antioxidative enzymes (catalase, peroxidase and superoxide dismutase). However, excess absorbtion of iron becomes toxic and can displace the cell redox balance toward a pro-oxidant state, causing alterations in the morphologic, biochemical and physiological characteristics of the plants, which contribute in ROS formation via the Fenton reaction (Hell and Stephan, 2003). Li *et al.* (2009) were reported that embryonic and adult medaka *Oryzias latipes* exposed to nano-iron show lipid peroxidation and alterations in antioxidant enzyme activity.

2.3.4 Cadmium (Cd)

Cadmium is one of the most important environmental contaminants hence limited availability for plant uptake because of low solubility in soils (McBride, 1994). Cd^{+2} is a non-redox metal promote indirectly generation of active oxygen species (AOS) (Sandalio *et al*., 2001; Schu¨tzendu¨bel *et al*., 2001; Olmos *et al*., 2003). High concentration of Cd^{+2} causes physiological, biochemical and genetical changes that lead to plant phyto-toxicity, and hence growth decrease (Liao *et al*., 2005; Nouairi *et al.*, 2006; Siroka *et al.*, 2004).

It has been demonstrated that Cd^{+2} increased lipid peroxidation in *Phaseolus vulgaris* roots and leaves (Chaoui *et al.*, 1997), *Helianthus annuus* leaves (Gallego *et al.*, 1996), *Pisum sativum* shoot and root tissues (Lozano-Rodriguez *et al.*,1997) and Oryza sativa leaves (Chien *et al.*, 2002).

.

2.3.5 Chromium (Cr)

Chromium is a very prevalent toxic element in contaminated soil (Kimbrough, 1999; Molnár, 1989). Cr is one of the heavy metal pollutants produced from ferrochrome, tanning and pigment industries. In nature, Cr exists in two different stable oxidation states; trivalent (Cr^{III}) and hexavalent (Cr^{VI}) chromium. Both Cr^{III} and Cr^{VI} differ in terms of mobility, bioavailability and toxicity. Cr^{VI} is found to be more toxic than Cr^{III} (Panda and Patra, 1997).

Cr is harmful to human health acting as cumulative poison when enters the food chain (Lahouti and Peterson, 1979; Peralta-Videa, *et al.*, 2009; Zayed *et al*., *1998).* Cr^{IV} is known to be carcinogenic for humans, and harmful effects of chromium on DNA have been described in fish (WHO, 1988).

Cr toxicity in plants can trigger an accumulation of lipid peroxides, oxidize proteins, and cause oxidative destruction (Shanker *et al.*, 2005).

2.3.6 Mercury (Hg)

Mercury is an important pollutant of water worldwide. It diffuses to the environment as a result of human activities such as silver and gold mining, coal combustion and dental amalgams (Luoma and Rainbow, 2008b).

Organic methyl mercury and inorganic (mercurous, mercuric) forms exist in nature. Organic forms are the result of methylation of inorganic mercury by microorganisms in sediments and water. Methylmercury is generally more toxic to fish than the inorganic forms (Houserova *et al*., 2006). Studies suggest that both organic and inorganic forms of mercury participate in the formation of ROS (Larose *et al*., 2008; Mieiro *et al*., 2010) by inhibiting the activities of antioxidative enzymes especially glutathione reductase (Mtthofer *et al*., 2004).

Due to its transition properties, mercury is readily uptaken by plants, accumulates at high level, results in toxicity or even death of plants (Boening, 2000; Patra and Sharma, 2000; Esteban *et al*., 2008).

2.3.7 Copper (Cu)

Copper plays a significant role in many physiological processes, including photosynthesis, respiration, carbohydrate distribution, nitrogen fixation, protein and cell wall metabolism, antioxidant activity and hormone perception in plants. At the cellular level, copper is a structural

and catalytic component of many proteins and enzymes involved in a variety of metabolic pathways (Pilon *et al*., 2006).

Plants usually find an ample supply of copper in soils, but it must be maintained at low concentrations, because copper at high concentrations can be a stress factor triggering physiological responses (Yruela, 2005).

It has been previously reported that an excess of Cu can result in production of ROS and free radicals. These substances can damage cell membranes by binding to the sulfhydryl groups of membrane proteins or by increasing rates of lipid peroxidation (Liu *et al*., 2004).

2.3.8 Aluminium (Al)

Aluminium is one of the most abundant elements in the Earth' crust (Kochian, 1995). Recent research suggests that at least part of Al toxicity is due to the oxidative stress caused by this ion. Therefore, the tolerance of plants to Al may be related to the activity of the plants' antioxidant systems (Sharma and Dubey, 2007; Giannakoula *et al*., 2010; Panda and Matsumoto, 2010; Ma *et al*., 2012; Xu *et al*., 2012). This system involves enzymatic and non-enzymatic antioxidant mechanisms for removal of ROS produced during oxidative stress (Gratão *et al*., 2005).

Al has pro-oxidant activity (Exley, 2004), enhancing production of ROS and changing the redox state of the metabolic system in cells (Achary *et al*., 2008; Ma *et al*., 2012; Xu *et al*., 2012). Even in the presence of toxic levels of Al, the levels of ROS in plant tissues do not significantly change in tolerant plants (Giannakoula *et al*., 2010).

2.3.9 Nickel (Ni)

Nickel (Ni) is an essential micronutrient; it acts as the active center of the urease enzyme required for nitrogen metabolism in higher plants.

Many studies have been pointed to Ni toxicity symptoms in plants such as inhibition of growth, photosynthesis, mineral nutrition, sugar transport and water relations (Seregin and Kozhevnikova, 2006).

Ni has ability to produce $OH^{\cdot}$ via a Fenton/Haber–Weiss reaction (Kehrer, 2000). However, Ni does not seem to be an efficient catalyst of this reaction due to its relatively high oxidation/reduction potential (Leonard *et al*, 2004). It has been shown that Ni-dependent reduction of H_2O_2 leading to $OH^{\cdot}$ formation may be elevated by some chelating agents. ROS may be generated from the reactions catalyzed by NADPH oxidases (Sagi and Fluhr, 2006). SOD, POD and CAT play a main role in regulation the rate of ROS-generating and scavenging enzymes under nickel stress (Yan *et al*., 2008).

2.3.10 Zinc (Zn)

Zinc is necessary for many physiological processes (Rengel, 1999). Zn is a prime industrial pollutant of the global and aquatic environment (Barak and Helmke, 1993). It is extremely toxic at high concentrations and can inhibit plant growth and disrupt various essential physiological processes (Clemens, 2006), lower respiratory rate, increase membrane damage in sunflower plants (Ismail and Azooz, 2005),slow down metabolic activity and induce oxidative damage in various plant species (Panda *et al*., 2003). However, because of the similarities in ion radius of divalent cations, excess zinc can shift certain physiological equilibrium by local competition at various sites (Kemper, 1997).

Plants deals with heavy metal ion stress in different ways including exclusion and chelation as well as expression of stress protein genes. Increases in both proline and lipid peroxidation levels with increasing Zn concentration are indicative of a correlation between ROS generation (hydroxyl radicals mostly) and ROS scavenging by proline (Matysik *et al*., 2002). It was demonstrated that exposure of rice cells and duckweed to Zn can enhance the intracellular level of H_2O_2 and ROS (Lin *et al.*, 2005).

2.3.11 Cobalt (Co)

Cobalt (Co) is a trace element that can be a contaminant as a result of agricultural additives or metal refineries (Bakkaus *et al*., 2005). It has been known that Co is an essential element for humans, animals and prokaryotes but a physiological function for this element in higher plants has not been identified (Parmer and Chanda, 2005).

Certain plant species have the ability to uptake metals (such as Co) from soils, thus, cleaning the environment. Co is necessary for the normal metabolic functions of the plant, but at excess level of this metal is toxic and may severely interfere with physiological and biochemical functions causing irreversible damage to a number of vital metabolic constituents of plant cell and cell membrane (El-Sheekh *et al*., 2003; Jayakumar and Vijayarengan, 2006).

2.4 Plants

2.4.1 *Petroselinum crispum*

Parsley have a number of medicinal properties including, antimicrobial, (Wong and Kitts, 2006) antianemic, menorrhagic, anticoagulant, antihyperlipidemic, antihepatotoxic (Ozturk *et al*., 1991) antioxidant (Nielsen *et al*., 1999) and laxative (Kreydiyyeh *et al*., 2001). It has been used to treat lumbago, as a blood pressure regulator, to treat eczema, knee, ache, impotence and nose bleed (Manderfeld *et al*., 1997). Parsley seed are also used as a diuretic and the hypoglycemic activity of parsley has been shown by Ozsoy *et al*. (2006).

Parsley has revealed the presence of several classes of flavonoids (*Fejes et al, 2000*) which about 100 mg/100 g fresh weight of parsley, it is supposed that the health promoting effect of parsley may be attributed to its flavonol compounds (Hall *et al*., 1990). The biological actions of flavonoids are may be due to antioxidant activity, enzyme inhibition, and the capacity to scavenge free radicals (Lin. *et al*., 2002; Potapovich and Kostyuk, 2003). Components of fresh parsley leaf scavenge superoxide anion in vitro (Campanella *et al*., 2003). Supplementation of diets with fresh parsley leaf can increase antioxidant capacity of rat plasma (Hempel *et al*., 1999).

2.4.2 *Eruca sativa*

Rocket (*Eruca sativa*) is a dark green annual plant, about 20 to 50 cm in height, with a spicy-pungent taste (Morales and Janick, 2002). Many studies have shown that many plants of the Brassicaceae family contain phytochemicals such as flavonoids and glycosinolates (Jin *et al*., 2009; Bogani and Visioli, 2007; Schaffer *et al*., 2005) which have beneficial health effects such as cancer prevention (Ambrosone and Tang, 2009). In addition, it has been reported rocket has a stringent, diuretic, digestive, emollient, depurative, laxative, rubefacient, tonic, stomachic, anti-inflammatory for colitis and stimulant properties (Yaniv *et al*., 1998; Bianco, 1994).

In cosmetics rocket has been used for promoting hair regrowth, the treatment of oily scalp and as a facial tonic (Ellison *et al*., 1980). Rocket is an easy plant to grow, so, it consider best choice by researchers as experimental material (Germ and Osvald, 2005).

Rocket extract significantly scavenged several ROS and RNS. It has an effective antioxidant and renal protective action and stop oxidative damage inflicted to the kidney (Gurpreet. *et al*., 2007).

2.4.3 *Rosmarinus officinalis L.*

Among the herbal extracts reported to have antioxidant activity, rosemary is one of the most widely commercialized plant extracts. It is used as a culinary herb for flavoring and as an antioxidant in processed foods and cosmetics (Zheng and Wang, 2001). Related to its antioxidant and anti-inflammatory properties, several phenolic compounds have been extracted, mainly carnosic and rosmarinic acids (Alfonso and Sant’Ana, 2008; Pérez-Fons *et al*., 2010).

Rosemary extracts formulations are the only ones commercially available for use as antioxidants in the European Union and the United States, and they are marketed in an oil-soluble form, as a dry powder, and in water-dispersible or water-miscible formulations (Aguilar, *et al*., 2008, Bozˇin *et al*., 2007). Rosemary scavenges superoxide radicals, repress lipid

oxidation and chelate metals. The phenolic content of rosemary is ~150 mg/g (Peschel *et al*., 2007).

Antioxidants from our diet play an important role in helping endogenous antioxidants for the neutralization of oxidative stress (Lien *et al.*, 2008). The nutrient antioxidant deficiency is one of the causes of many chronic and degenerative pathologies as well as the aging process and some acute pathologies (trauma and stroke). Each nutrient is unique in its structure and antioxidant function (Ceriello, 2008).

Chapter Three
Material and Methods

3.1 Materials:

3.1.1 Plant samples

Fresh Plant samples (*Petroselium cripsum* and *Eruca Sativa*) have been obtained from market, *Rosmarinus officinalis* collected from local garden of Mutah university between June-November, 2013 (Table 1).

Table (1)
Classification of selectable plants.

Common Name	Scientific Name	Botanical family
Garden Rocket	*Eruca Sativa*	Brassicacae
Parsley or garden parsley	*Petroselium cripsum*	Apiaceae
Rosemary	*Rosmarinus officinalis*	Lamiaceae

3.1.2 Chemicals:

The chemicals used in this research were analytical grade and shown in table (2).

Table (2)
The chemicals used in this research.

Chemicals	Molecular formula	Company, Country
Cobaltus chloride	$CoCl_2$	AVONDALE Laboratories, England
Cupric sulfate	$CuSO_4$*$5H_2O$	FULKA, Spain
Aluminium sulfate 2-hydrate	$Al(SO_4)_2$•$2H_2O$	F.E.R.O.S.A, England
Potassium chromate	CrK_2O_4	FULKA, Spain
Lead IIV acetate 3- hydrate	$Pb(CH_3CO_2)_2$ $3H_2O$	AVONDALE laboratory, England
Ammoniummolybdate	$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$.	Laboratory rasayan, India
Iron IIV sulfate hydrogen	$Fe(HSO_4)_3$	BDH Technical laboratory supplies, England
Zinc sulfate	$ZnSO_4$	FULKA, Spain
Cadmium nitrate 4-hydrate	$Cd(NO_3)_2$.$4H_2O_2$	Laboratory rasayan, India
Nickel chloride	$NiCl_2$	SIGMA, USA
Mercuric chloride	Hg_2Cl_2	Laboratory rasayan, India
Phenol reagent (folin and ciocalteu)	$C_{10}H_2NaO_5S$	Laboratory rasayan, India
Pyrogallol	$C_6H_6O_3$	LCN biomedicals inc., Shanghai
Sulphuric acid 98%	H_2SO_4	Laboratory rasayan, India
Sodium dehydrogenate	NaH_2po_4.H_2O	Laboratory rasayan, India
Di Sodium Hydrogen phosphate	Na_2HPO_4	FULKA, Spain
Sodium Flouride	NaF	BDH laboratory supplies, England
Catechol	$C_6H_4(OH)_2$	BDH laboratory supplies, England
Potasium Sodium(+)-Tartrate GLR	$KNaC_4H_4O_6{\cdot}4H_2O$.	PHARMACOS LTD, England
Hydrogen peroxides (6%wt/v)	H_2O_2	Laboratory rasayan, India
Sodium carbonate anhydrous	Na_2CO_3.	FULKA, Spain
Cupric sulfate anhydrous	$CuSO_4$	FULKA, Spain

3.1.3 Instruments:

The Instruments used in this research were shown in table (3).

Table (3)

The instruments used in this research.

Instruments	Company, Country
Centrifuge	Sigma 112, West Germany
Spectophotometer	UV/VIS, Biological Engineering Manangement Co. LTD. UK

3.2 Methods:

3.2.1 Enzyme Extraction:

3.2.1.1 Crude Enzyme (Peroxidase) extract.

Leaves of the selected plants were homogenized in 50 mM Sodium phosphate buffer (pH 6.8) in the ratio 1:1 (w/v)) in a blender for 3 min. The homogenate was filtered using cloth sheet and then was centrifuged at 16,000 rpm for 20 min at room tempreture.

The supernatant was collected as crude enzyme solution and was kept at 4^0C until use (Hammerschmidt and Kuc, 1982).

3.2.1.2 Crude Enzyme (Polyphenol oxidase) extract.

Leaves of the selected plants were homogenized in 10 mM sodium fluoride solution in the ratio1:5 (w/v) in a blender for 3 min. The homogenate was filtered using cloth sheet , and then was centrifuged for 10 min. at 16,000 rpm. The supernatant was collected as crude enzyme solution and was kept at 4^0C until use (Mustafa *et al*., 2007).

3.2.2 Enzyme assay:

3.2.2.1 Measurement of the activity of peroxidase (POX, EC 1.11.17).

Peroxidase activity was measured using the method described by Kumar and Khan (1982). The assay mixture of POX contained 2 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H_2O_2 and 0.5 ml of the enzyme extract. The solution was incubated for 5 min at 25 °C after which the reaction was terminated by adding 1 ml of 2.5 M H_2SO_4. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5 M H_2SO_4 at zero time.

The activity was expressed in unit/mg protein. One U is defined as the change in the absorbance per one min per mg protein (Kumar and Khan, 1982).

3.3.2.2 Measurement of the activity of Polyphenol oxidase (PPO, EC 1.10.3.1).

Polyphenol oxidase (PPO) activity was assayed by the method of Kumar and Khan (1982). Assay mixture for PPO contained 2 ml of 0.1 M phosphate buffer (pH 6.0), 1 ml of 0.1 M catechol and 0.5 ml of enzyme

extract. Then was incubated for 5 min at 25 ^oC, after which the reaction was stopped by adding 1 ml of 2.5 N H_2SO_4. The absorbance of the purpurogallin formed was read at 495 nm. To the blank, 1ml of 2.5 N H_2SO_4 was added at zero time of the same assay mixture. PPO activity was expressed in U/mg protein (Parimala and Muthuchelian, 2011).

3.4 Protein estimation

Total protein concentration was determined in triplicate by Lowry *et al.* (1951) method using bovine serum albumin as a standard. The amount of the soluble protein was calculated from the standard curve as mg of protein per ml of test sample.

The mixture was freshly prepared and contained per tube : 4.9 ml of sodium carbonate (2%) dissolved in 0.1 M NaOH, 0.05 ml of sodium potassium tartrate (2.7 %) and 0.05 ml of copper sulfate (1%).

Table (4)
Lowry method

Reagent added	Tubes Number									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10 (extract)**
B S A (1 mg/ml)	00	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.5
H_2O ml	0.5	0.45	0.4	0.35	0.30	0.25	0.2	0.15	0.1	00
Reagent mixture ml	5	5	5	5	5	5	5	5	5	5

Each tube was mixed well, then incubated exactly 10 min at room tempreture. After incubation time, 0.05 ml fresh dilution of the stock Folin –Ciocalteu (phenol reagent:water 1:1)(v/v) was added to all tubes. Then the tubes were incubated for 30 min at room tempreture. The absorbancy of all tubes were read at 700 nm against the blank.

The absorbance reading for each tube was used to make a standard curve and to find out the protein amount of crude extract enzymes (Peroxidase and polyphenol oxidase) extract of rosemary, parsley and rocket as shown in table (4).

3.5 Kinetic Determination

POX activity was measured with H_2O_2 (substrate) at varying concentrations (1-20 mM) at 420 nm**.** PPO was measured with catechol (substrate) at varying concentrations (1-20 mM) at 495 nm. Lineweaver-Burk double reciprocal plots were used to to calculate the enzyme kinetic parameters: V_{max}, K_m and K_i values. Calculation was determined by plotting 1/V versus 1/[S] in the absence or presence of selected heavy metals (Cd^{2+}, Co^{2+}, Al^{3+} Cr^{2+}, Cu^{2+}, Fe^{3+}, Mo^{2+}, Zn^{2+}, Hg^{2+}, Ni^{2+} and Pb^{2+}) at 400µM concentration.

3.6 Heavy Metats solutions

Fresh stock solutions of different heavy metals (Cd^{2+}, Co^{2+}, Al^{3+} Cr^{2+}, Cu^{2+}, Fe^{3+}, Mo^{2+}, Zn^{2+}, Hg^{2+}, Ni^{2+} and Pb^{2+}) of 1M were prepared (Gao *et al.,* 2008). Different concentrations were used (200, 400, 800 µM). Maximum effects were seen at concentration 400 µM. Therefore, for all experiments, 400 µM concentration of heavy metal was used.

3.7 Specific activity (Unit/mg).

The specific activity was calculated by dividing V_{max} value by concentration of protein for each selected plant.

3.8 Relative activity (%)

The relative activity (%) of crude enzyme extract was caculated by dividing V_{max} value of the treated sample (with heavy metal) by V_{max} value of control sample (not treated) multiplied by 100%.

3.9 Statistical analysis:

For all experiments, samples of the selected plants were analyzed and all the assays were carried out in triplicate . The results were expressed as mean ± standard deviation using Microsoft excel 2007.

Chapter Four
Results and Discussion

Metalloenzymes comprise approximately one-third of the known enzymes and require stoichiometric quantities of transition metal ions for their catalytic activities. Peroxidases are important detoxifying enzymes serving to eliminate cells of excess H_2O_2 under normal and stress conditions (Ellis., 2006). Peroxidases in plants are mainly haem-containing enzymes where the prosthetic group is protoporphyrin IX (Laloue *et al*., 1997), So it is consider as a metalloenzyme contains a tightly bounds metals which are needed to maintain the structural integrity of enzyme and participate in electrophilic catalysis (Ellis., 2006).

Polyphenoloxidases are enzymes, belonging to a group of copper containing metalloproteins and are members of oxidoreductases, that catalyze the oxidation of a wide range of phenolic compounds by utilizing molecular oxygen (Queiroz, *et al.* 2008, Simsek and Yemenicioglu 2007).

It was reported that peroxidases remain active in the presence of a number of metal ions, but recent reports have indicated their inhibition by certain metal ions (Keyhani *et al.*, 2003).

In the present study, the kintetic parameters of POX and PPO in crude extract of leaves of rocket, rosemary and parsley in presence and absence of various heavy metals were investigated.

4.1 Protein content

The protein content in the crude enzyme extracts of leaves of rosemary, parsley and rocket were measured by lowry method using BSA as standard protein. The result showed that the crude extract of leaves of rosemary has the highest amount of protein (2.46 mg/ml), while rocket leaves have the lowest amount of protein (0.22 mg/ml) as listed in table (5).

Table (5)
Protein content (mg/ml) in the crude ezyme (POX and PPO) extracts of rosemary, parsley and rocket.

Selected Plants	Protein amount (mg/ml) Mean ±SD, (n=3)
Rosmarinus officinalis	2.46 ±0.247
Petroselium cripsum	0.24 ±0.038
Eruca stavia	0.22 ±0.6

4.2 Effect of heavy metals on POX and PPO activity in rosemary, parsley and rocket extracts.

All results are summarized in tables from 6 to 11 and will be analyzed according to their effects: Uncompetitive inhibition, noncompetitive and activation.

A- Uncompetitive inhibition.

Uncompetitive inhibitor binds to the enzyme-substrate complex and it effectively reduces the concentration of that complex by converting some of it into the ternary EIS complex. The effect of inhibitor realized on increasing the amount of substrate which binds to the enzyme, giving an apparent increase in enzyme-substrate affinity and a decrease in K_m values. As the inhibitor is not competed out by large amounts of substrate, quite the opposite as it needs substrate to bind to the enzyme first, it is effective at high substrate concentrations and therefore decreases V_{max} values. Once the inhibitor has bound to enzyme, it will prevent it from turning the substrate into product by direct interaction, or due to a change in conformation of the active site (Voet & Voet, 2011). Therefore, the ratio of V_{max}/K_m of the crude enzyme extract will be affected. The V_{max}/K_m ratio is called the "catalytic power" (Rocha *et al.*, 1998) and is a good parameter for finding the most effective or ineffective heavy metal (Baritaux *et al.*, 1991).

It was found that Cd^{2+}, Ni^{2+} and Cu^{2+} have an uncompetitive inhibition on the crude enzyme(POX) extract acivity in all selected plants by decreasing both K_m and V_{max} values. Simillar effects were seen in the crude enzyme (POX) extract activity of rosemary by $Hg^{2+}$and Co^{2+}, of parsley by Mo^{2+}, Al^{3+}, Pb^{2+}, Cr^{2+}, Zn^{2+} and Hg^{2+}, and of rocket by Mo^{2+} and Al^{3+} (Tables from 6 to 8).

According to Cd^{2+}, it may inhibit or promote the activities of antioxidant enzymes involved in the oxidative defense system (Ahmad *et al.*, 2011). It was reported by Radeva *et al.* (2010) that antioxidant enzymes including peroxidase, polyphenyl oxidase and catalase activities in pea seedlings were gradually increased by increasing the concentrations of Cd^{2+}.

K_i defined as an equilibrium constant for the inhibitor binding to the enzyme, which reflects the strength of the interaction between the enzyme and the inhibitor (Voet & Voet, 2011). So, in the absence of inhibitor K_i=0, low K_i value mean tight binding between enzyme and inhibitor and high K_i value mean weak binding. It was clearly noted that low K_i value in the uncompetitive inhibition reflects strong binding between the heavy metals and the enzyme (Tables from 6 to 11).

Recent research revealed that heavy metals such as Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+} and Pb^{2+} as well as some organic and inorganic compounds such as sodium azide, cyanide, L–cystine, dichromate, ethylenethiourea, hydroxylamine, sulfide, vanadate, p–aminobenzoic acid are well-known inhibitors of horseradish peroxidase (Nomngongo., *et al* 2008).

Regarding PPO, uncompetitive inhibition was shown in the crude enzyme extract of rosemary in the presence of Cu^{2+} and Pb^{2+} by decreasing

both K_m and V_{max} values . Simillar effects were seen in the crude enzyme (PPO) extract activity of rocket by Zn^{2+}.

It was clearly noted that K_i value in an uncompetitive inhibition is strong reflecting by K_i value (Tables 9, 10, 11).

B- **Noncompetitive inhibition**

Noncompetitive inhibitors bind to an inhibitor site which is remote from the active site. They inhibit the enzyme by causing a conformational change which prevents enzyme from converting substrate to product. The substrate and inhibitor are capable of binding to the enzyme at the same time to create a ternary complex. A classical noncompetitive inhibitor (pure noncompetitive) has no effect on substrate binding, so the enzyme-substrate affinity not changed. A mixed inhibitor allows the substrate to bind, but reduces its affinity, so the K_m value is increased. At high substrate concentrations, noncompetitive inhibitors of both classical and mixed types reduce V_{max} value. Moreover, they will lower the catalytic power at low substrate concentrations and as aresult they will decrease the ratio of V_{max}/K_m (Tables from 6 to 11) (Shengwen *et al*., 2013).

K_m is the concentration of substrate at which enzyme activity is half-maximum. Its value includs not only the affinity of the substrate for the enzyme but also the rate at which the enzyme-bound substrate is converted to the product in the catalytic reaction. Thus, K_m value can be interpreted as a crude measurement of the affinity of the substrate for the enzyme (Shengwen *et al*., 2013 ; Amine *et al.*, 2006).

As shown in table (6) the V_{max} value of POX decreased and its k_m value may increase or unchanged which illustrated that no effect on the enzyme-substrate affinity under effect of these heavy metals. It was found that Fe^{3+}, Al^{3+} and Pb^{2+} were acted as a mixed NCI on the crude enzyme (POX) extract of rosemary by decreasing V_{max} value from 0.11umol/min (control) to 0.02, 0.07 and 0.035 umol/min, and increasing K_m value from 4 mM (control) to 4.99, 7.76 and 5 mM respectively. Moreover, Cr^{2+} and Co^{2+} have the same effects on the crude enzyme (POX) extract of rocket only.

Incontrast, Mo^{2+} was the only HM that exhibit a pure NCI in the crude enzyme extract of rosemary (K_m value unchange, V_{max} value decreased).

The ratio of V_{max}/K_m which reflects the specificity of enzyme to substrate, it was found to be $27.5*10^{-3}$, $42*10^{-3}$ and $38*10^{-3}$in rosemary, parsley and rocket, respectively. Moreovere, catalytic power was decreased under noncompetitive inhibitors, and it reached the lowest value in crude ezyme (POX) extracts of rosemary and rocket with Fe^{3+} ($4*10^{-3}$, $3*10^{-3}$), respectively.

Comparing specific activity of the crude enzyme (POX) extracts (control) in the absence of treatment by HM ($140*10^{-3}$), ($44*10^{-3}$) and

($227*10^{-3}$ unit/mg) for rosemary, parsley and rocket with the specific crude enzyme extract treated with HM revealed that specific activity was decreased in all types of inhibition (Tables 6, 7 and 8).

Tables (6 and 7) showed that K_i value in mixed non competitive inhibition is high relatively (compared to uncompetitive inhibition) that reflects the loose binding between the enzyme and the inhibitor.

One of the characteristics of noncompetitive inhibitors, that they can work at high and low substrate concentrations, which decrease the ratio of V_{max}/K_m (Tables 6 and 8) (Shengwen *et al*., 2013).

As shown in table (9) the V_{max} value of crude enzyme (PPO) extract decreased and its k_m value may be increased or unchanged which illustrate that no effects on the enzyme-substrate affinity under the treatment of these heavy metals. It was found that Zn^{2+} and Mo^{2+} were acted as a low mixed NCI on the crude enzyme (PPO) extract of rosemary by decreasing V_{max} value from 0.057umol/min (control) to 0.05 and 0.055 umol/min, and increasing K_m value from 0.87 mM (control) to 1.1and 3.4 mM, respectively. Moreover, Cr^{2+} and Hg^{2+} have the same effects on the crude enzyme (PPO) extract of parsley (Table 10).

According to the ratio of V_{max}/K_m which reflects the specificity of enzyme to substrate, It was found to be $65*10^{-3}$, $136 *10^{-3}$ and $10*10^{-3}$in rosemary, parsley and rocket, respectively. Moreovere, catalytic power decreased under noncompetitive inhibition, it reached the lowest value in crude enzyme (PPO) extract from rosemary treated with Mo^{2+} ($16*10^{-3}$) and parsley treated with Hg^{2+} ($5.7 *10^{-3}$) (Tables 9 and 10).

Many studies have shown that Hg-induced toxicity in plants results from the binding of its ionic forms (Hg^{2+}) to SH- groups of proteins, disruption of structure, and displacement of essential elements (Van Assche and Clijsters, 1990; Hall, 2002; Schützendübel and Polle, 2002).

Comparing specific activity of crude enzyme (PPO) extracts (control) in the absence of treatment by HM ($72.5*10^{-3}$), ($15*10^{-3}$) and (32 unit/mg) of rosemary, parsley and rocket with the crude enzyme (PPO) extracts treated with HM revealed the decrease in specific activity in all types of inhibition (Tables 9, 10 and 11).

It was reported that, both redox-active metals (Fe^{3+},Cu^{2+},Cr^{2+}) and redox-inactive metals (Pb^{+2}, Cd^{+2}, Hg^{+2}) may rise the production of ROS species including hydroxyl radical ($HO^{\cdot}$), superoxide radical ($O^{\cdot}_2$-) or hydrogen peroxide (H_2O_2) (Ercal *et al*., 2001) and eventually, such rise in ROS species inactivates antioxidant enzymes including peroxidases, catalases, superoxide dismutases that responsible for free radical detoxification (Dietz *et al*., 1999 and Sahw *et al*., 2004).

C- Activation

Since many enzymes contain sulfhydral (-SH), alcohol, or acid groups as part of their active sites, any chemical which can react with them acts as an irreversible inhibitor. Heavy metals such as Ag^{2+}, Hg^{2+}, Pb^{2+} have strong affinities for -SH groups ,The nucleophilic nature of the thiol group is also important in the formation of mercaptide bond with metals and for reacting with selected electrophiles (Foyer and Noctor, 2005; Mullineaux and Rausch, 2005).

Metalloenzymes, require many quantities of metal ions as cofactors, typically transition metal ions, for their catalytic activities. The roles of metal ions in enzyme active sites (aside from structure maintenance) include electron transfer, oxygen atom transfer, formation of coordinated hydroxide, electrophilic catalysis, as well as substrate binding (Ellis., 2006). The presence of an electron donor first results in the formation of an enzyme substrate complex, and then in the oxidation of the electron donor. The electron donor provides the driving force in the continuing catalysis of H_2O_2, while its absence effectively stops the reaction.

Figures (6,7 and 8) showed that Cr^{2+} was acted as an activator for the crude enzyme (POX) extracts of rosemary (Relative activity 154.5%) when compared to the control (Relative activity 100%). On the other hand, the same effects of Zn^{2+}(Relative activity 132%) and Hg^{2+}(Relative activity 133%) on the crude enzyme (POX) extract of rocket , as well as Fe^{+3} (Relative activity 700%) and Co (Relative activity 118%) on the crude enzyme (POX) extracts of parsley.

The results showed that the relative activity of crude enzyme (PPO) in the selected plants were increased in the presence of 400 µM of Cd^{2+}, Co^{2+}, Al^{3+}, Fe^{3+} and Ni^{2+} (Figure 9, 10, 11) and also evidenced by the decreased in the K_m and the increase in the V_{max} values (Tables 9,10,11). Meanwhile, the relative activity of crude enzyme (PPO) extracts of parsley and rocket was increased in presence of Cu^{2+},Mo^{2+} and Pb^{2+}. Incontrast, it was found that Zn^{2+} acted as an activator only on the crude enzyme (PPO) in parsley(Figure 10). On the other hand, Hg^{2+} and Cr^{2+}has the same effects on crude enzyme (PPO) extracts of rockets and rosemary (Figure 9, 11).

Cr^{2+} stress can cause induction and activation of superoxide dismutase (SOD) and of antioxidant catalase at lower concentrations whereas at higher concentrations the SOD activity did not increase further and catalase activity decreased (Shanker *et al*., 2003a; Gwozdz *et al*., 1997). Also antioxidant enzymes activity (POX and PPO) was increased with an increase in the Co^{2+} level of soil (Jayakumar *et al*., 2007).

Table (6)
Kinetic parameters of crude enzyme (POX) extracts of *Rosmarinus officinalis* in control and in presence of different heavy metals. Mean ±SD (n=3).

Kinetic parameters

Heavy metals	K_m(mM)	V_{max}(umol /min)	Ki (µM)	V_{max}/ K_m	Specific activity (unit/mg)	Effects
Control	4	0.11	0	27.5*10-3	140*10-3	Normal
Cd	3	0.067	-1.6*10-3	22*10-3	87*10-3	Un competitive
Zn	2.6	0.027	-1.1*10-3	10*10-3	34*10-3	Un competitive
Cr	1.6	0.17	- 0.6*10-3	106*10-3	216*10-3	Activation
Ni	1.7	0.01	- 0.47*10-3	5.8*10-3	12*10-3	Un competitive
Hg	0.69	0.016	- 0.48*10-3	23*10-3	20*10-3	Un competitive
Co	0.57	0.023	- 0.46*10-3	40*10-3	30*10-3	Un competitive
Fe	4.99	0.02	1.6*10-3	4*10-3	25*10-3	Mixed non competitive
Cu	0.5	0.0035	- 0.45*10-3	7*10-3	4.5*10-3	Un competitive
Al	7.76	0.07	0.4*10-3	9*10-3	90*10-3	Mixed non competitive
Pb	5	0.035	1.6*10-3	7*10-3	44*10-3	Mixed non competitive
Mo	3.8	0.036	-1*10-3	9*10-3	45*10-3	Pure non competitive

Table (7)
Kinetic parameters of crude enzyme (POX) extracts of *Petroselium cripsum* in control and in presence of different heavy metals. Mean ±SD (n=3).

Kinetic parameters

Heavy metals	K_m(mM)	V_{max}(umol /min)	Ki (µM)	V_{max}/ K_m	Specific activity (unit/mg)	Effect
Control	2.6	0.11	0	$42*10^{-3}$	$44*10^{-3}$	Normal
Cd	1.7	0.07	$-1.1*10^{-3}$	$41*10^{-3}$	$28*10^{-3}$	Un competitive
Zn	1.97	0.046	$-1.6*10^{-3}$	$23*10^{-3}$	$18.7*10^{-3}$	Un competitive
Cr	1.16	0.047	-0.7*10-3	40*10-3	19*10-3	Un competitive
Ni	0.88	0.06	$- 0.6*10^{-3}$	$68*10^{-3}$	$24*10^{-3}$	Un competitive
Hg	0.266	0.0055	$-0.4*10^{-3}$	$20*10^{-3}$	$2*10^{-3}$	Un competitive
Co	4.4	0.13	$0.57*10^{-3}$	$29*10^{-3}$	$53*10^{-3}$	Activation
Fe	0.77	0.2	$-0.4*10^{-3}$	$730*10^{-3}$	$59*10^{-3}$	Activation
Cu	1.35	0.05	$-0.8*10^{-3}$	$37*10^{-3}$	$20*10^{-3}$	Un competitive
Al	0.5	0.06	$-0.49*10^{-1}$	$120*10^{-3}$	$24*10^{-3}$	Un competitive
Pb	0.95	0.06	$-0.6*10^{-3}$	$63*10^{-3}$	$24*10^{-3}$	Un competitive
Mo	0.49	0.04	$-4.8*10^{-3}$	$81*10^{-3}$	$16*10^{-3}$	Un competitive

Table (8)
Kinetic parameters of crude enzyme (POX) extracts of *Eruca Sativa* in control and in presence of different heavy metals. Mean ±SD (n=3).

			Kinetic parameters			
Heavy metals	**K_m(mM)**	**V_{max}(umol /min)**	**Ki (µM)**	**V_{max}/ K_m**	**Specific activity (unit/mg)**	**Effect**
Control	1.3	0.05	0	$38*10^{-3}$	$227*10^{-3}$	Normal
Cd	0.8	0.005	$- 1*10^{-3}$	$6*10^{-3}$	$2.7*10^{-3}$	Un competitive
Zn	0.66	0.066	$-0.8*10^{-3}$	$100*10^{-3}$	$300*10^{-3}$	Activation
Cr	1.13	0.008	$- 3*10^{-3}$	$7*10^{-3}$	$36*10^{-3}$	Mixed non competitive
Ni	0.48	0.009	$-0.6*10^{-3}$	$18.75*10^{-3}$	$40*10^{-3}$	Un competitive
Hg	3.9	0.066	$0.2*10^{-3}$	$17*10^{-3}$	$300*10^{-3}$	Activation
Co	1.67	0.012	$1.3*10^{-3}$	$7*10^{-3}$	$50*10^{-3}$	Mixed non competitive
Fe	13.4	0.04	$0.0\ 4*10^{-3}$	$3*10^{-3}$	$180*10^{-3}$	Mixed non competitive
Cu	0.7	0.023	$- 0.8*10^{-3}$	$33*10^{-3}$	$100*10^{-3}$	Un competitive
Al	0.38	0.003	$- 0.5*10^{-3}$	$3.5*10^{-3}$	$4*10^{-3}$	Un competitive
Pb	2.5	0.036	$0.4*10^{-3}$	$14*10^{-3}$	$160*10^{-3}$	Mixed non competitive
Mo	0.53	0.02	$-0.6*10^{-3}$	$37*10^{-3}$	$90*10^{-3}$	Un competitive

Tabel (9)
Kinetic parameters of crude enzyme (PPO) extracts of *Rosmarinus officinali*s in control and in presence of different heavy metals Mean ±SD (n=3).

			Kinetic parameters			
Heavy metals	**K_m(mM)**	**V_{max}(umol /min)**	**Ki (µM)**	**V_{max}/ K_m**	**Specific activity (unit/mg)**	**Effect**
Control	0.87	0.057	0	$65*10^{-3}$	$72.5*10^{-3}$	Normal
Cd	0.4	0.09	0. 7*10-3	307*10-3	114*10-3	Activation
Zn	1.1	0.05	1.5*10-3	45*10-3	63*10-3	Mixed non competitive
Cr	0.8	0.06	-0.4*10-3	75*10-3	76*10-3	Activation
Ni	8.6	0.15	- 0.2*10-3	17*10-3	190*10-3	Activation
Hg	4.9	0.2	0.086*10-3	40*10-3	254*10-3	Activation
Co	2.3	0.09	0.25*10-3	39*10-3	114*10-3	Activation
Fe	4.4	0.11	0.1*10-3	25*10-3	140*10-3	Activation
Cu	0.5	0.016	-0.9*10-3	32*10-3	20*10-3	Un competitive
Al	2.6	0.09	0.2*10-3	34*10-3	114.5*10-3	Activation
Pb	0.38	0.022	- 0.7*10-3	57*10-3	27*10-3	Un competitive
Mo	3.4	0.055	0.14*10-3	16*10-3	70*10-3	Mixed non competitive

Table (10)

Kinetic parameters of crude enzyme (PPO) extracts of *Petroselium cripsum* in control and in presence of different heavy metals Mean ±SD (n=3).

•

Heavy metals	Kinetic parameters					
	K_m(mM)	V_{max}(umol /min)	Ki (µM)	V_{max}/K_m	Specific activity (unit/mg)	Effect
Control	0.28	0.038	0	136*10-3	15*10-3	Normal
Cd	0.21	0.039	- 1.3*10-3	136*10-3	15*10-3	Activation
Zn	1.26	0.08	0.1*10-3	63.5*10-3	32*10-3	Activation
Cr	2.75	0.028	0.045*10-3	10*10-3	11*10-3	Mixed non competitive
Ni	1	0.11	0.16*10-3	110*10-3	44.5*10-3	Activation
Hg	1.94	0.011	0.067*10-3	5.7*10-3	4.5*10-3	Mixed non competitive
Co	0.57	0.07	0.4*10-3	123*10-3	28*10-3	Activation
Fe	0.87	0.047	0.2*10-3	54*10-3	19*10-3	Activation
Cu	0.63	0.04	0.32*10-3	63.5*10-3	16*10-3	Activation
Al	2	0.14	0.066*10-3	70*10-3	56.7*10-3	Activation
Pb	4.36	0.05	0.027*10-3	11.5*10-3	20*10-3	Activation
Mo	1.28	0.07	0.1*10-3	54.7*10-3	28*10-3	Activation

Tabel (11)

Kinetic parameters of crude enzyme (PPO) extracts of *Eruca Sativa* in control and in presence of different heavy metals. Mean ±SD (n=3).

Heavy metals	Kinetic parameters					
	K_m(mM)	V_{max}(umol /min)	Ki (µM)	V_{max}/K_m	Specific activity (unit/mg)	Effect
Control	0.7	0.007	0	$10*10^{-3}$	$32*10^{-3}$	Normal
Cd	2.87	0.011	$0.13*10^{-3}$	$4*10^{-3}$	$50*10^{-3}$	Activation
Zn	0.52	0.003	$- 1.5*10^{-3}$	$6*10^{-3}$	$13.6*10^{-3}$	Un competitive
Cr	2.15	0.013	$0.2*10^{-3}$	$6*10^{-3}$	$59*10^{-3}$	Activation
Ni	0.25	0.019	$-0.6*10^{-3}$	$76*10^{-3}$	$86*10^{-3}$	Activation
Hg	0.9	0.078	$1.3*10^{-3}$	$86.6*10^{-3}$	$354*10^{-3}$	Activation
Co	0.6	0.03	$-2.6*10^{-3}$	$50*10^{-3}$	$136*10^{-3}$	Activation
Fe	6.35	0.0096	$0.0 5*10^{-3}$	$1.5*10^{-3}$	$44*10^{-3}$	Activation
Cu	1.11	0.01	$0.68*10^{-3}$	$9*10^{-3}$	$45*10^{-3}$	Activation
Al	6.3	0.033	$0.0 5*10^{-3}$	$5*10^{-3}$	$150*10^{-3}$	Activation
Pb	0.24	0.022	$-0.6*10^{-3}$	$92*10^{-3}$	$100*10^{-3}$	Activation
Mo	1.35	0.04	$0.4*10^{-3}$	$30*10^{-3}$	$181*10^{-3}$	Activation

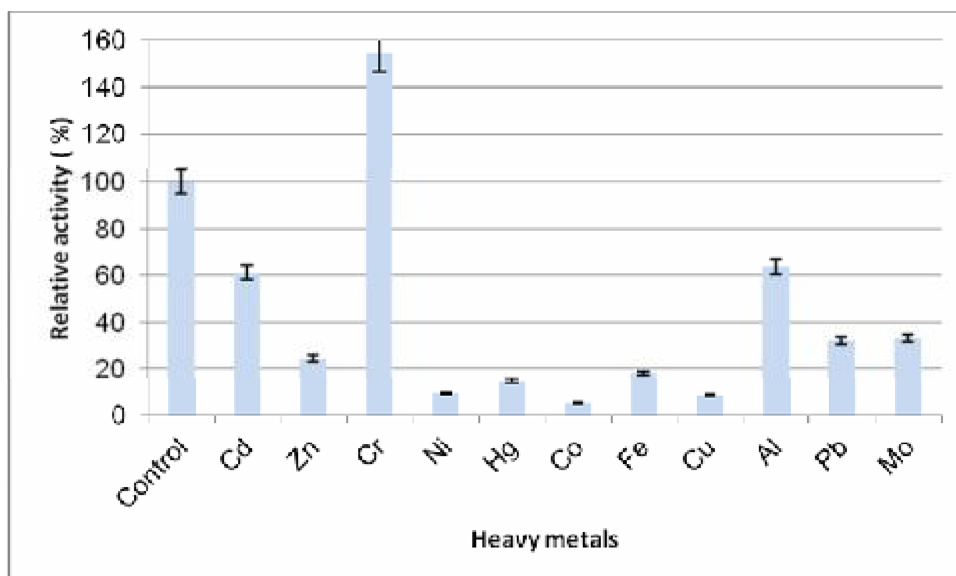


Figure (6)
Relative activity (%) of crude enzyme (POX) extracts of *Rosmarinus officinalis* in control and in the presence of different heavy metals. Mean ±SD (n=3).

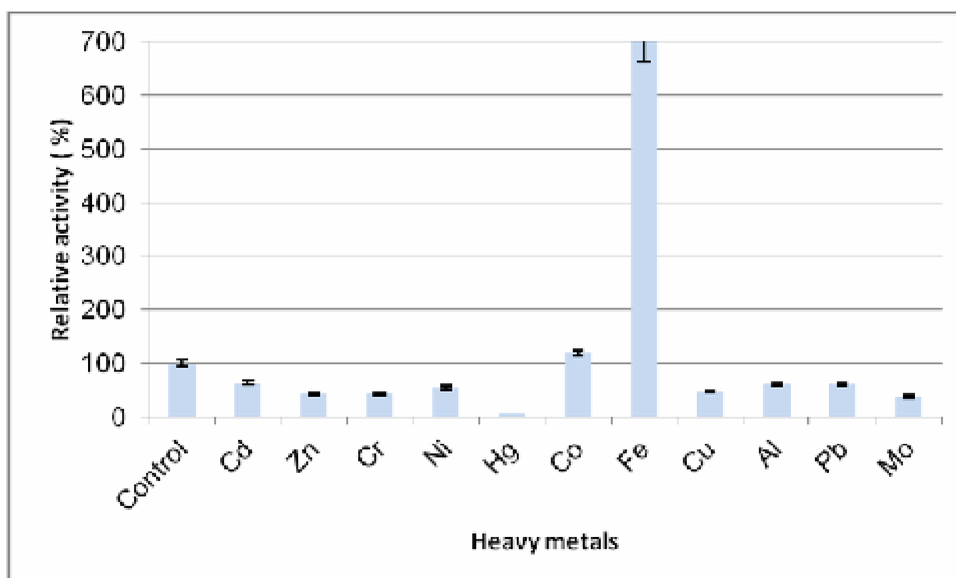


Figure (7)
Relative activity (%) of crude enzyme (POX) extracts of *Petroselium cripsum* in control and in the presence of different heavy metals. Mean ±SD (n=3).

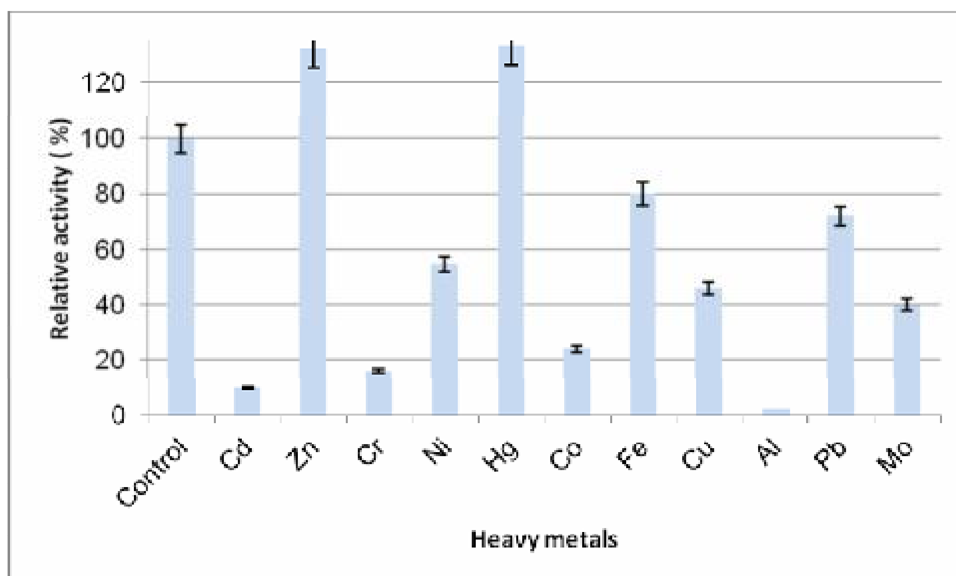


Figure (8)
Relative activity (%) of crude enzyme (POX) extracts of *Eruca Sativa* in control and in the presence of different heavy metals. Mean ±SD (n=3).

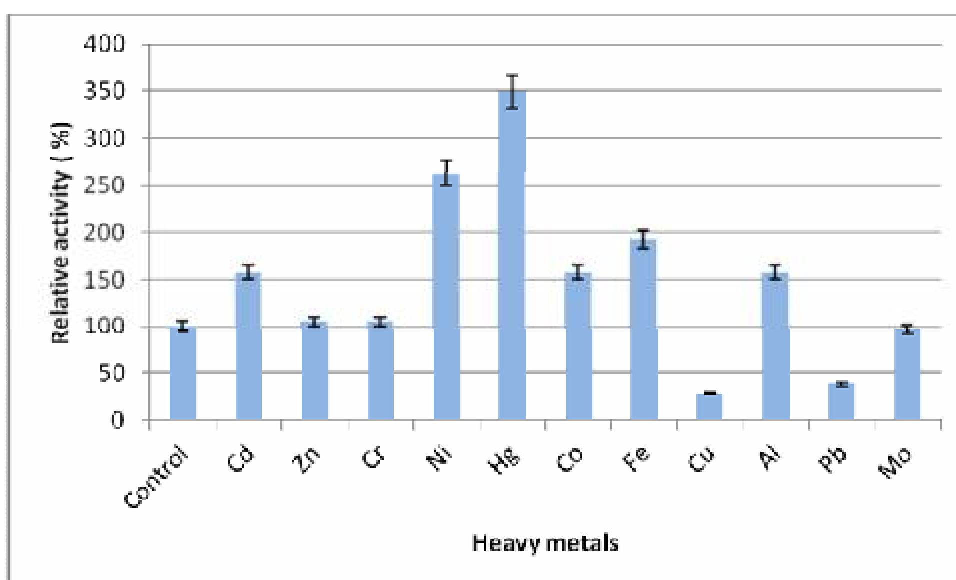


Figure (9)
Relative activity (%) of crude enzyme (PPO) extracts of *Rosmarinus officinalis* in control and in the presence of different heavy metals. Mean ±SD (n=3).

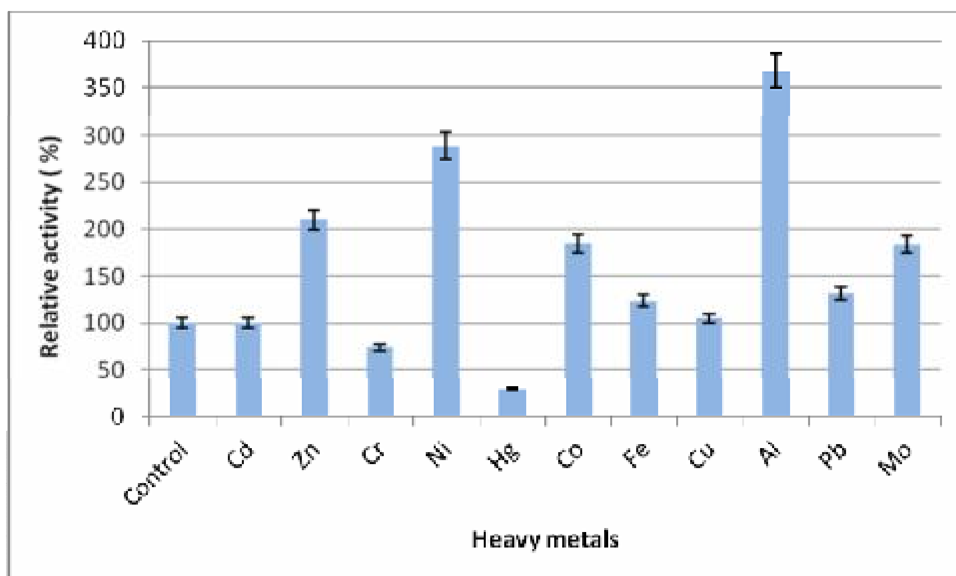


Figure (10)
Relative activity (%) of crude enzyme (PPO) extracts of *Petroselium cripsum* in control and in the presence of different heavy metals. Mean ±SD (n=3).

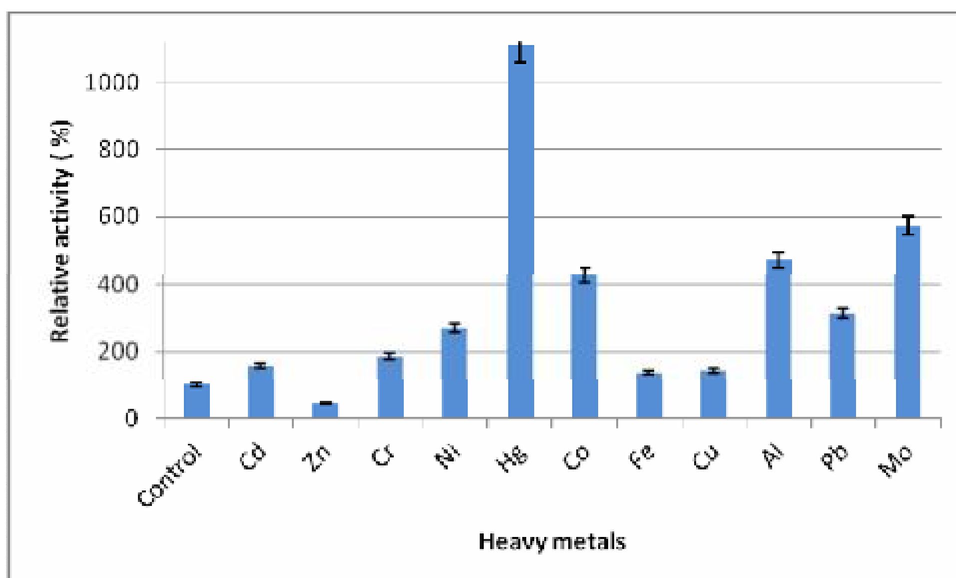


Figure (11)
Relative activity (%) of crude enzyme (PPO) extracts of *Eruca Sativa* in control and in the presence of different heavy metals. Mean ±SD (n=3).

4.3 Conclusion

Plant green leaves of *Petroselium cripsum, Rosmarinus officinalis and Eruca Sativa* were analyzed for POX and PPO activities.

Results demonstrated that the crude enzymes (POX, PPO) extracts of the selected plants have a potential activities according to the K_m and V_{max} values.

Presence of heavy metals altered these activities by acting as a uncompetitive or noncompetitive inhibitors or acting as activators depending on the type of heavy metals. For example, Cd^{2+}, Co^{2+}, Al^{3+}, Fe^{3+} and Ni^{2+} acted as activator in all selected plants, while uncompetitive inhibition were observed in presence of Cu^{2+} and Pb^{2+} for PPO from rosemary as evidenced by the decrease in the K_m and the V_{max} values, and $Hg^{2+}$and Cr^{2+}acted as a noncompetitive inhibitors in the crude enzyme (PPO) extracts of parsley. Furthermore, Fe^{3+}, Al^{3+}, Pb^{2+} and Mo^{2+} were found to act as a noncompetitive inhibitors on the crude enzyme (POX) extract of rosemary, while Cr^{+2}, Co^{2+}, Fe^{3+} and Pb^{2+} acted as a noncompetitive inhibitors on the crude enzyme (POX) extract of rosemary.

4.4 Recommendation

Furthere experiments are needed for isolation and purification of these enzymes from *Petroselium cripsum, Rosmarinus officinalis and Eruca Sativa*.

References

Achary, V.M.; Jena, S.; Panda, K.K. & Panda, B.B. (2008). Aluminum induced oxidative stress and DNA damage in root cells of Allium cepa L. Ecotox**. Environ. Saf.** 70: 300-310.

Adonaylo, V.N. & Oteiza, P.I. (1999). "Pb promotes lipid oxidation and alterations in membrane physical properties," **Toxicology**, vol.132, no.1: 19–32.

Aguilar, F.H.; Autrup, S.; Barlow, L.; Castle, R. & Crebelli, W. et. al. (2008). Use of rosemary extracts as a food additive: scientific opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food. **EFSA J.** 721: 1–29.

Ahmad, P.; Nabi, G. & Ashraf, M. (2011). Cadmium-induced oxidative damage in mustard [Brassica juncea (L.) Czern. & Coss.] plants can be alleviated by salicylic acid. **S. Afr. J. Bot.** 77: 36–44.

Ahn, D.U. & Nam, K.C. (2007). Effects of ascorbic acid and antioxidants on color, lipid oxidation and volatiles of irradiated ground beef. Rad. **Physics Chem.** 71(1-2): 151-156.

Alasoumi, S.; Al-Howiriny, T.A; Al-Yahya, M. & Rafatullah, S. (2008). Gastroprotective effects of radish "raphanus sativus" L. on experimental gastric ulcer models in Rats. **FARMACIA**. 46: 204-214.

Alfonso, M.S. & Sant'Ana, L.S. (2008). Effects of pretreatment with rosemary (Rosmarinus officinalis L.) in the prevention of lipid oxidation in salted tilapia fillets. **J. Food Quality**, 31: 586-595.

Al-Sereitia, M.R.; Abu-Amerb, K.M. & Sena, P. (1999). Pharmacology of rosemary (Rosmarinus officinalis Linn.) and its therapeutic potentials. **Indian Journal of Experimental Biology,** Vol.37: 124-131.

Ambrosone C. B. and Tang L (2009). Cruciferous vegetable intake and cancer prevention: Role of nutrigenetics. **Cancer Prev. Res**., (Phila Pa), 2: 298–300.

Amine, I., K. Norazaidah and K.I.E. Hainida,(2006). Antioxidant activity and phenolic content of raw and blanched Amaranthus species. **Food Chem**., 94: 47-52.

Antioxidant properties of Mediterranean food plant extracts:

Antosiewicz, D. and M. Wierzbicka. 1999. Localization of lead in Allium cepa L., cell by electron microscopy. **Journal of Microscopy**, 195: 139-146.

Bakkaus, E.B.; Gouget, J.P.; Gallien, H.; Khodja, F.; Carrot, J.L. & Morel, R. Collins. (2005). Concentration and distribution of cobalt in higher plants: The use of micro-PIXE spectroscopy. **Nuclear Physics Research,** B231: 350-356.

Barak, P. & Helmke, P.A. (1993). **The Chemistry of Zink. In: Proceedings of the International Symposium on Zinc in Soils and Plants,** Robson, A. (Ed.). Kluewer Acad. Press, Perth, Australia, 90-106.

Baranowska –Morek A. (2003) Roślinne mechanizmy tolerancjina toksyczne działanie metali ciężkich. **Kosmos** 52,283.

Baritaux, O.; Amiot, M.J.; Richard, H. & Nicolas, J. (1991). Enzymatic browning of basil (Ocimum basilicum L.) studies on phenolic compounds and polyphenoloxidase. **Sciences des Aliments,** 11: 49-62.

Becana, M.; Moran. J.F. & Ormaetxe, I. (1998). Iron–dependent oxygen free radical generation in plants subjected to environmental stress: toxicity and antioxidant protection. **Plant Soil**, 201: 137–147.

Bhattachrjee, S. (2005). Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transduction in plant. **Current Science,** 89, 1113-1121.

Bianco, V.V. (1994). Rocket, an Ancient Underutilized Vegetable Crop and It's Potential. Rocket Genetic Resources Network, Report of the First Meeting, Lisbon, Portugal. **Biomarkers Prev**., 17: 938–944.

Boening, D.W. (2000). Ecological effects, transport and fate of mercury: a general review. **Chemosphere**, 40, 1335–1351.

Bogani P, Visioli F (2007). Antioxidants in the Mediterranean diets: An update. **World Rev. Nutr. Diet**., 97: 162–179.

Bozˇin, B.; Mimica-Dukic´, N.; Samojlik, I. & Jovin, E. (2007). Antimicrobial and antioxidant properties of rosemary and sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils. **J. Agric. Food Chem**. 55: 7879–7885.

Briat, J.F. & Lobre´aux, S. (1997). Iron transport and storage in plants. **Trends Plant Sci.,** 2: 187–193.

Cai, B.S., Y.Z. Mei Sun and H. Corke (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. **J. Agric. Food Chem**. 53(2):7749-7759.

Campanella, L, A Bonanni, G Favero and M Tomassetti (2003) Determination of antioxidant properties of aromatic herbs, olives and fresh fruit using an enzymatic sensor. **Analyt Biochem**, 375: 1011-1016.

Ceriello A. (2008). Possible role of oxidative stress in the pathogenesis of hypertension. Review. **Diabetes Care.** 31(Suppl 2): S181-4.

Chaoui, A.; Mazhouri, S.; Ghorbal, M.H. & Ferjani, E.E. (1997). Cadmium and Zinc induction of lipid peroxidation and effects of antioxidant enzyme activities in bean (Phaseolus vulgaris L.). **Plant Sci.** 127: 139–147.

Chatterjee, M.; Saluja, R.; Kanneganti, S. & et. al. (2007). Biochemical and molecular evaluation of neutrophil NOS in spontaneously hypertensive rats. **Cell Mol. Biol.**;53:84-93.

Chien H.F.; Lin, C.C.; Wang, J.W.; Chen, C.T. & Kao, C.H. (2002). Changes in ammonium ion content and glutamine synthetase activity in rice leaves caused by excess cadmium are a consequence of oxidative damage. **Plant Growth Regul.** 36: 41–47.

Chisari, M.; Barbagallo, R.N. & Spagna, G. (2007). Characterization of polyphenol oxidase and peroxidase and influence on browning of cold stored strawberry. **Journal of Agriculture and Food Chemistry** 55: 3469-3479.

Clemens, S. (2001). Molecular mechanisms of plant metal tolerance and homeostasis. **Planta**, 212: 475.

Clemens, S. (2006). Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. **Biochimie**, 88: 1707-1719

Dalton, T.D.; Shertzer, H.G. and Puga, A. (1999) Regulation of gene expression by reactive oxygen. Annu. Rev. **Pharmacol.** 39, 67–101.

Dat J, Vandenabeele S, Vranová E, Van Montagu M, Inzé D, Van Breusegem F (2000) Dual action of the active oxygen species during plant stress responses. **Cell Mol Life Sci** 57: 779–795

De Vos, C.H.R.; Vonk, M.J.; Vooijs, R. & Schat, H. (1992). Glutathione depletion due to copper-induced phytochelatin synthesis causes oxidative stress in Silene cucubalus. **Plant Physiol.,** 98: 853-858.

Del Río, L.A.; Corpas, F.J.; Sandalio, L.M.; Palma, J.M.; GÓmez, M. & Barroso, J.B. et al. (2002). Reactive oxygen species, antioxidant systems and nitric oxide in peroxisomes. **Journal of Experimental Botany,** 53 (372), 1255-1272.

Desikan, R.; Hancock, J.T. & Neill, S.J. (2003). Oxidative stress signaling. In H. Hirt and Shinozaki K (eds.), Plant responses to abiotic stress: topic in current genetics. **Springer-Berlag, Berlin, Heidelberg, Nes York**. pp.121-148.

Dietz, K.J.; Bai, E.R. & KRA MER, U. (1999). Free radicals and active oxygen species as mediators of heavy metal toxicity in plants. In: Prasad M.N.V., **Hagemeyer J.** (Eds.), Heavy Metal Stress in Plants. From Molecule to Ecosystems. Springer, Berlin, pp 73–97.

Ellis Walther R. (2006). Metalloenzymes. **Encyclopedia of Molecular Cell Biology and Molecular Medicine**, Published Online.

Ellison, J.A.; Hylands, P.; Peterson, A.; Pick, C.; Sanecki, K. & Stuart, M. (1980). **Enciclopedia delle erbe.** 1st. edn. A. Mondadori, Verona.

El-Sheekh, M.M; El-Naggar, A.H.; Osman, M.E.H. & El-Mazaly, E. (2003). Effect of cobalt on growth, pigment and the photosynthetic

electron transport in Monoraphidium minutum and Nitzhia perminuta. **Brazilian Journal of Plant Physiology**, 15: 159-166.
enhanced peroxidase activity with induced systemic resistance

Ercal N, Gurer-Orhan H, Aykin-Burns N (2001): Toxic metals and oxidative stress part I: Mechanisms involved in metal induced oxidative damage. **Current Topics in Medicinal Chemistry** 1, 529–539.

Esteban, E.; Morenoa, E.; Pe˜nalosa, J.; Cabrero, J.I.; Mill, N.M. & Zornoza, P. (2008). Short and long-term uptake of Hg in white lupin plants: Kinetics and stress indicators. **Environ. Exp. Bot.** 62: 316–322.

Exley, C. (2004). The pro-oxidant activity of aluminum. **Free. Radic. Biol. Med.** 36: 380-387.

Fejes, S, A Blazovics, E Lemberkovics, G Petri, E Szoke and A Kery,

Fennema, O.R. (1996). **Food chemistry**. 3rd ed. New York: Marcel Dekker. 1067 p.

Fernandez Donna E. and Su-Chiung Fang (2002) Effect of Regulated Overexpression of the MADS Domain Factor AGL15 on Flower Senescence and Fruit Maturation . **Plant Physiology** vol. 130 no. 1 78-89.

Foyer, C.H. & Noctor, G. (2005). Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. **Plant Cell**, 17, 1866–1875.

Freire, R. S., N. Duran and L. T. Kubota (2002). Development of laccase based flow injection electrochemical biosensor for the determination of phenolic compounds and its application for monitoring remediation of Kraft E1 paper mill effluent., **Anal. Chim. Acta.,** 463: 229-238.

Fukao, T. & Bailey-Serres, J. (2004). Plant responses to hypoxia - is survival a balancing act? Trends in Plant Science 9: 449-456.

Gallego, S.M.; Benavides, M.P. & Tomaro, M.L. (1996). Effect of heavy metal ion excess on sunflower leaves: Evidence for involvement of oxidative stress. **Plant Sci.,** 121: 151-159
geographical differences. **J. Physiol. Pharmacol**., 56 (Suppl. 1): 115–124.

Germ, M.; Kreft, I. & Osvald, J. (2005). Influence of UV-B exclusion and selenium treatment on photochemical efficiency of photosystem II, yield and respiratory potential in pumpkins (Cucurbita pepo L.). **Plant Physiol. Biochem**., 43: 445-448.

Giannakoula, A.; Moustakas, M.; Syros, T. & Yupsanis, T. (2010) Aluminum stress induces up-regulation of an efficient antioxidant system in the Al-tolerant maize line but not in the Al-sensitive line. **Environ. Exp. Bot.** 67: 487-494.

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. **Plant Physiol Biochem** 48:909–930.

Gratão, P.L.; Prasad, M.N.V.; Cardoso, P.F.; Lea, P.J.; Azevedo, R.A. (2005). Phytoremediation: green technology for the clean up of toxic metals in the environment. **Brazilian Journal of Plant Physiology** 17: 53-64.

Gurpreet-Singh, Jalota, S.K., and Yadvinder-Singh. (2007). Manuring and residue management effects on physical properties of a soil under the rice-wheat system in Punjab, India. **Soil and Tillage Research**, 94: 239-238.

Gwozdz, E.A.; Przymusinski, R.; Rucinska, R. & Deckert, J. (1997). Plant cell responses to heavy metals: molecular and physiological aspects. **Acta Physiol Plant,** 19: 459–465.

Halder, J., P. Tamuli and A. N. Bhaduri (1998). Isolation and characterization of polyphenol oxidase from Indian tea leaf (Camellia sinensis). **The Journal of Nutritional Biochemistry**., 9(2): 75-80

Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King MC (1990).Linkage of early-onset familial breast cancer to chromosome17q21.**Science.** 250(4988):1684–1689.

Hall, J.L. (2002). Cellular mechanisms for heavy metal detoxification and tolerance. **J. Exp. Bot.** 53: 1–11.

Halliwell, B. & Gutteridge, J.M.C. (2007). **Free radicals in biology and medicine.** 4th. Oxford, UK: Clarendon Press.

Halliwell, B. (2006). Reactive species and antioxidants: redox biology is a fundamental theme of aerobic life. **Plant Physiol.,** 141: 312–322.

Halliwell, B. (2007). Biochemistry of oxidative stress. **Biochem. Soc. Trans.** 35: 1147–1150.

Hammerschmidt, R., E.M. Knuckles and J. Kuc, 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to Colletotrichum lagenarium. **Physiol. Plant Pathol**.,20: 73-82.

Hasanuzzaman Mirza *et al*, (2013) Enhancing Plant Productivity Under Salt Stress: Relevance of Poly-omics. **Salt Stress in Plants**. pp 113-156.

Hatz, S., Lambert, J.D.C. & Ogilby, P.R. (2007). Measuring the Lifetime of Singlet Oxygen in a Single Cell: Addressing the Issue of Cell Viability. **Photochemical & Photobiological Sciences,** Vol. 6, No. 10, pp. 1106-1116.

Heber, U.; Bukhov, N.G.; Shuvalov, V.A.; Kobayashi, Y. & Lange, O.L. (2001). **J. Exp. Bot.** 52, 1999-2006

Hell, R. & Stephan, U.W. (2003). Iron uptake, trafficking and homeostasis in plants. **Planta,** 216: 541–551.

Hempel, J, H Pforte, B Raab, W Engst, H Bohm and G Jacobash,(1999). Flavonols and flavones of parsley cell suspension culture change the antioxidative capacity of plasma in rats. **Nahrung**, 43: 201-204

Hill, H.Z. (1992). The function of melanin or six blind people examine an elephant. Bio. **Essays**. 14: 49–56.

Hossain, V.H.; Tervahauta, A.I.; Schat, H. & Karenlampi, S.O. (2011). Plant metallothioneins - metal chelators with ROS scavenging activity? **Plant Biology,** Vol.13, No.2, 225-232.

Houserova, P.; Kuban, V.; Spurny, P. & Habarta, P. (2006). Determination of total mercury and mercury species in fish and aquatic ecosystems of Moravian rivers. **Veterinarni Medicina,** 51: 101–110.

Inze, D. & Van, M.G.M. (1995). Oxidative stress in plants. **Curr. Opin. Biotech.** 6, 153.

Ismail, A.M. & Azooz, M.M. (2005). Effect of zinc supply on growth and some metabolic characteristic of safflower and sunflower plants. Ind**. J. Plant Physiol.,** 10: 260-266.

Jaleel, C.A.; Manivannan, P.; Lakshmanan, G.M.A.; Sridharan, R. & Panneerselvam, R. (2007c). NaCl as a physiological modulator of proline metabolism and antioxidant potential in Phyllanthus amarus. **Comptes Rendus Biologies**. 330: 806–813.

Jaleel, C.A.; Manivannan, P.; Sankar, B.; Kishorekumar, A.; Gopi R.; Somasundaram, R. & Panneerselvam, R. (2007b). Water deficit stressmitigation by calcium chloride in Catharanthus roseus; effects on oxidative stress, proline metabolism and indole alkaloid accumulation. **Colloids and Surfaces B: Biointerfaces**. 60: 110–116.

Jayakumar, K. & Vijayarengan, P. (2006). Alterations in the carbohydrate metabolism of Vigna mungo (L.) Hepper as affected by cobalt stress. **Indian Journal of Environment and Ecoplanning**, 3: 18-21.

Jayakumar, K., C.A. Jaleel and P. Vijayarengan,(2007). Changes in growth, biochemical constituents and antioxidant potentials in radish (Raphanus sativus L.) under cobalt stress. **Turkish J. Biol.,** 31: 127-136.

Jin J, Koroleva OA, Gibson T, Swanston J, Magan J, Zhang Y, Rowland IR, Wagstaff C (2009). Analysis of phytochemical composition and chemoprotective capacity of rocket (Eruca sativa and Diplotaxis tenuifolia) leafy salad following cultivation in different environments **J. Agric. Food Chem.,** 57(12): 5227-5234.

John, R.; Ahmad, P.; Gadgil, K. & Sharma, S. (2008). Effect of cadmium and lead on grpwth, biochemical parameters and uptake in Lemna polyrrhiza L. **Plant Soil Environ.,** 54: 262-270.

Joo Young Kim et al. (2001) Two polyphenol oxidases are differentially expressed during vegetative and reproductive development and in response towounding in the Fuji apple_ **Plant Science** 161 1145–1152.

Kehrer, J.P. (2000). The Haber-Weiss reaction and mechanisms of toxicity. **Toxicol.,** 149: 43–50.

Kemper, H.; Gorres, M. & Frenzel, B.1 (997). Ti and Pb concentrations in rainwater-fed bogs in Europe as indicators of past anthropogenic activities. **Water Air Soil. Poll.,** 100: 367-377

Keyhani, J.; Keyhani, E.; Einollahi, N.; Minai-Tehrani, D. & And Zarchipour, S. (2003). Heterogeneous inhibition of horseradish peroxidase activity by cadmium. **Biochim. Biophys. Acta.** 1621:140-148.

Khan, A.G.; Ku Ek, T.M.; Chau Dhury, T.M.; Khoo, C.S., & Hayes, W.J. (2000). Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. **Chemosphere**, 41: 197.

Kimbrough, D.E.; Cohen, Y.; Winer, A.M.; Creelman, L. & Mabuni, C. (1999) .A critical assessment of chromium in the environment. **Crit. Rev. Env. Sci.** Tec. 29: 1-46.

Kochian, L.V. (1995). Cellular mechanisms of aluminum toxicity and resistance in plants. Annu. Rev. **Plant Physiol. Plant Mol. Biol.** 46, 237–260.

Kondo, K.; Kurihara, M. & Fukuhara, K. (2001). Mechanism of antioxidant effect of catechins. **Methods in Enzymol.** 335, 203-217.

Kreydiyyeh SI, Usta J, Kaouk I, et al (2001). The mechanism underlying the laxative properties of parsley extract. **Phytomedicine**, 8, 382-8.

Kumar, K.B. & Khan, P.A. (1982). Peroxidase in excised ragi (Eleusine coracana Cv. PR. 202) leaves during senescence, **Indian Journal of Experimental Botany**, Vol.20, No.8, pp.412-416.

Kupper, H.; Parameswaran, A.; Leitenmaier, B.; Trtilek, M. & Setlik, I. (2007). Cadmium-induced inhibition of photosynthesis and long-term acclimation to cadmium stress in the hyperaccumulator Thlaspi caerulescens. **New Phytol.,** 175: 655-674.

Lahouti, M. & Peterson, P.J. (1979). Chromium accumulation and distribution in crop plants. **J. Sci. Food Agr.** 30: 136-142.

Laloi, D., Richard, M., Lecomte, J., Massot, M., Clobert, J.,(2004). Multiple paternity in clutches of common lizard Lacerta vivipara: data from microsatellite markers. **Mol. Ecol.** 13, 719–723.

Laloue, H.; Weber-Lofti, F.; Lucau-Danila, A. & Guillemat, P. (1997). Identification of ascorbate and guaiacol peroxidase in needle chloroplasts of spruce trees. **Plant Physiol. Biochem.** 35, 341-346.

Larose, C.; Canuel, R.; Luccote, M.D. & Giulio, R. (2008). Toxicological effects of methylmercury on walleye (Sander vitreus) and perch (Perca flavescens) from lakes of the boreal forest. **Comparative Biochemistry and Physiology** C, 147: 139–149.

Lee, M.Y.; Lee, M.K. & Park, I. (2007). Inhibition of onion extract on polyphenol oxidase and enzymatic browning of taro (Colocasia antiquorum var. esculenta). **Food Chemistry,** 105: 528-532.

Leonard, S.S.; Harris, G.K. & Shi, X. (2004). Metal-induced oxidative stress and signal transduction. **Free Rad. Biol. Med,** 37: 1921–1942.

Li, H.C.; Zhou, Q.; Wu, Y.; Fu, J.; Wang, T. & Jiang, G. (2009). Effects of waterborne nano-iron on medaka (Oryzias latipes): Antioxidant enzymatic activity, lipid peroxidation and histopathology. **Ecotoxicology and Environmental Safety**, 72: 3684–3692.

Liao, B.; Liu, H.; Zeng, Q.; Yu, P.; Probst, A. & Probst, J. (2005). Complex toxic effects of Cd^{2+}, Zn^{2+}, and acid rain on growth of kidney bean (Phaseolus vulgaris L). **Environ Int.,** 31, 891-895.

Lien, A.; Pham-Huy, H.H. (2008). Chuong Pham-Huyc, Free Radicals, Antioxidants in Disease and HealthInternational, **Journal of Biomedical Science**, 4(2), 89-96.

Lin CM, Chen CS, Chen CT, Liang YC and Lin JK (2002) Molecular modeling of flavonoids that inhibits XO Biochem. **Biophys. Res. Commun**. 294: 167 -172.

Lin, C.W.; Chang, H.B. & Huang, H.J. (2005). Zinc induces mitogen-activated protein kinase activation mediated by reactive oxygen species in rice roots**. Plant Physiol. Biochem.** 43: 963–968.

Liu, J.; Xiong, Z.T.; Li, T.Y. & Huang, H. (2004). Bioaccumulation and ecophysiological responses to copper stress in two populations of Rumex dentatus L. from copper contaminated and non-contaminated sites.Environ. **Exp. Bot.,** 52: 43–51.

Lopez, M.G.; Sanchez-Mendoza, I.R. & Ochoa-Alejo, N. (1999). Compartive study of volatile components and fatty acids of plants and in-vitro cultures of parsley Petroselinum crispum (Mill) nym ex hill. **J. Agric. Food Chem.** 47: 3292–3296.

Lowry, O. H.; Rosebrough, N. J.; Farr, A.L. & Randall, R.J. (1951). **J. Biol Chem.**, 265-275.

Lozano-Rodriguez, E.; Hernandez, L.; Bonay, P. & Charpena-Ruiz, R. (1997). Distribution of cadmium in shoot and root tissue of maize and pea plants: physiological disturbances. **J. Exp. Bot.** 48: 123–128.

Lugasi, A.; Dworschak, E. & Hovari, J. (1995). Characterization of scavenging activity of natural polyphenols by chemiluminescence technique. Fed. European Chem. Soc. Proceed. of **Euro Food Chem**. VIII, Vienna, Austria, September 18-20. 3, 639-643.

Luoma, S.N. & Rainbow, P.S. (2008b). Methyl mercury. In: Metal Contamination in Aquatic Environments: **Science and Lateral Management.** Cambridge University Press, Cambridge. 368–392.

Lupea, A.X.; Pop, M. & Cacig, S. (2008). Structure radical scavenging activity relationships of flavonoids from Ziziphus and Hydrangea extracts. **Revista de Chimie.** 59(3), 309-313.

Lynn, A.; Collins, A.; Fuller, Z.; Hillman, K. & Ratcliffe, B. (2006). Cruciferous vegetables and colo-rectal cancer. **Proc Nutr Soc.** 65:135–144.

Ma, B.; Gao, L.; Zhang, H.; Cui, J. & Shen, Z. (2012). Aluminum-induced oxidative stress and changes in antioxidant defenses in the roots of rice varieties differing in Al tolerance. **Plant Cell Rep.** 31: 687-696.

Manerfeld, MM, HW Schafer, PM Davidson and EA Zottola, 1997. Isolation and identification of antimicrobial furocoum-arins from parsley. **J food Prot**, 60: 72-77.

Marshall, M.R.; Kim, J.M. & Wei, C.I. (2000). **Enzymatic browning in fruits, vegetables and seafoods.**

Matysik, J.; Alia, B.B. & Mohanty, P. (2002). Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants**. Curr. Sci.** 82: 525–532.

McBride MB. (1994). **Environmental chemistry of soils**. New York: **Oxford Univ Pr**. 406 p20, 73–82.

McCann SE (2008). Consumption of raw cruciferous vegetables is

Meharg AA (1993). The role of the plasmalemma in metal tolerance in angiosperms. **Physiol. Plant**. 88:191-198.

Mendel, R.R. (2005). "Molybdenum: biological activity and metabolism,

Mieiro CL, Ahmad I, Pereira ME, Duarte AC, Pacheco M (2010): Antioxidant system breakdown in brain of feral gulden grey mullet (Liza aurata) as an effect of mercury exposure. **Ecotoxicology** 19, 1034–1045.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. **Trends Plant Sci.** 7, 405-410.

Mittler, R.; Vanderauwera, S.; Gollery, M. & Breusegem, F.V. (2004). Abiotic stress series. Reactive oxygen gene network of plants. **Trends Plant Sci.** 9 (10): 490–498.

Mittler, R.; Vanderauwera, S.; Gollery, M. & Van Breusegem, F. (2004). Reactive oxygen gene network of plant. **TRENDS Plant Science** 9(10), 490-498.

Moller, M. (2001). Plant mitochondria and oxidative stress: Electron Transport, NADPH Turnover, and Metabolism of Reactive Oxygen Species. **Annual Review of Plant Physiology and Plant Molecular Biology,** 52(1), 561-591.

Molnár, L.; Fischer, E. & Kállay, M. (1989). Laboratory studies on the. effect, uptake and distribution of chromium in Eisenia foetida (Annelida, Oligochaeta). **Zool. Anz.** 223: 57-66.

Morales, M. and Janick, J. (2002). Trends in new crops and new uses. In Janick J. and Whipkey A. (ed) ASHS Press, Alexandria, VA.

Mtthofer, A.; Schulze, B. & Boland, W. (2004). Biotic and heavy metal stress response in plants: evidence for common signals. **FEBS Lett**. 566, 1.

Mullineaux, P. & Rausch, T. (2005). Glutathione, photosynthesis and the redox regulation of stress-responsive gene expression. **Photosynthesis Research,** 86, 459–474.

Mustafa Ümit Ünal, Aysun Şener and Kemal Şen (2007). Characterization of Sultaniye grape (Vitis vinifera L. cv. Sultana) polyphenol oxidase. **International Journal of Food Science & Technology,**Volume 42, Issue 9, pages 1123–1127.

Neill, S.J.; Desikan, R.; Clarke, A. & Hancock, J.T. (2002). Nitric oxide is a novel component of abscisic acid signaling in stomatal guard cells. **Plant Physiology,** 128, 13-16.

Nielsen, S.E., J.F. Young, B. Daneshvar, S.T. Lauridsen, P. Knuthsen, B. Sandstrom, & L.O. Dragsted, 1999. Effect of parsley (Petroselinum crispum) intake on urinary apigenin excretion, blood antioxidant enzymes and biomarkers for oxidative stress in human subjects. **British Journal of Nutrition**., 81: 447-455.

Nomngongo, P.N.; Catherine N.J.; Msagati, T.A.M.; Gumbi, B.P.; Iwuoha, E.I. (2012). **Physics and Chemistry of the Earth**, vol. 50-52. p: 252-261

Nouairi, I.; BenAmmar, W.; BenYoussef, N.; Daoud, D.B.; Ghorbal, M.H. & Zarrouk, M. (2006). Comparative study of cadmium effects on membrane lipid composition of Brassica juncea and Brassica napus leaves. **Plant Sci.,** 170, 511-519.

Olmos, E.; Martinez-Solano, J.R.; Piqueras A. & Hellin E. (2003). Early steps in the oxidastive burst induced by cadmium in cultured tobacco cells (BY-2 line). **J. Exp. Bot.** 54: 291–301.

Ozsoy-Sacan, O.; Yanardag, R.; Orak, H.; Ozgey, Y.; Yarat, A. & Tunali, T. (2006). Effects of parsley (Petroselinum crispum) extract

versus glibornuride on the liver of streptozotocin-induced diabetic rats. **J. Ethnopharmacol**. 104: 175-181.

Ozturk Y, Baser CHK, Aydin S.(1991) Hepatoprotectiventihepatotoxic) plants in Turkey. Proceedings of the 9th Symposium on Plant Drugs, Eskisehir, Turkey: 40-50.

Panda SK, Parta HK (1997) Physiology of chromium toxicity in plants – A Review. **Plant Physiol. Biochem**. 24(1):10-17.

Panda, S.K. & Matsumoto, H. (2010). Changes in antioxidant gene expression and induction of oxidative stress in pea (Pisum sativum L.) under Al stress. **BioMetals,** 23: 753-762.

Panda, S.K.; Chaudhury, I. & Khan, M.H. (2003). Heavy metals induce lipid peroxidation and affect antioxidants in wheat leaves. **Biol. Plant.,** 46: 289-294.

Papadakis, A.K.; Roubelakis-Angelakis, K.A. (2002). Oxidative stress could be responsible for the recalcitrance of plant protoplasts. **Plant Physiol Biochem**. 40, 549-559.

Parimala P., Muthuchelian K.(2011). Antioxidative responses and expression of insecticidal proteins in Bt cotton under high irradiance. **Journal of Stress Physiology & Biochemistry,** Vol. 7 No. 3 2011, pp. 201-211

Parmer, G. & Chanda, V. (2005). Effects of mercury and chromium on peroxidase and IAA oxidase enzymes in the seedlings of Phaseolus vulgaris. **Turkish Journal of Biology**, 2: 29-21

Patra, M. & Sharma, A. (2000). Mercury toxicity in plants. **Bot. Rev**. 66: 379–422.

Pei, Z.M.; Murata, Y.; Bnning, G.; Thomine, S.; Klusener, B. & Allen, G.J. et. al. (2000). Calcium channels activated by hydrogen peroxide mediate abscisic acid signaling in guard cell. **Nature,** 406, 731-734.

Peralta-Videa, J.R.; Lopez, M.L.; Narayan, M.; Saupe, G. & GardeaTorresdey, J. (2009). The biochemistry of environmental heavy metal uptake by plants: Implications for the food chain. **Int. J. Biochem. Cell B.** 41: 1665-1677.

Pérez-Fons, L.; Garzón, M.T. & Micol, V. (2010). Relationship between the antioxidant capacity and effect of rosemary (Rosmarinus officinalis L.) polyphenols on membrane phospholipid order. **J. Agric. Food Chem.,** 58: 161-171.

Peschel, W.; Dieckmann, W.; Sonnenschein, M. & Plescher, A. (2007). High antioxidant potential of pressing residues from evening primrose in comparison to other oilseed cakes and plant antioxidants. **Indust. Crops Prod.** 25(1), 44.

Pialis P, Saville BA (1998): Production of L-DOPA from tyrosine immobilized on nylon 6,6: enzyme stability and scale up. **Enzyme Microb Tech** 22: 261–268.

Queiroz, C.; Lopes, M.L.M.; Fialho, E. & Valente-Mesquita, V.L. (2008). Polyphenol oxidase: Characteristics and mechanisms of browning control. **Food Reviews International,** 24 (4):361-375.

Radeva, V.; Petrov, V.; Minkov, I.; Toneva, V. & Gechev, T. (2010). Effect of cadmium on Arabidopsis thaliana mutants tolerant to oxidative stress. **Biotechnol. Special Edition/On-Line,** 114-118.

Rai V.; Vaypay, E.E.P.; Sin, Gh, S.N. & Mehrotra, S. (2004). Effect of chromium accumulation on photosynthetic pigments, oxidative stress defence system, nitrate reduction, proline level and eugenol content of Ocimum tenuiflorum L . **Plant Sci.** 167, 1159.

Rasmusson, A.G.; Heiser, V.; Zabaleta, E.; Brennicke, A. & Grohmann, L. (1998). Physiological, biochemical and molecular aspects of Mitochondria complex in plants [J]. **Biochmi Biophys.** Acta. 1364, 101-111.

Rauser, W.E.; Schupp, R. & Rennenberg, H. (1991). Cysteine, γ-glutamylcysteine and glutathione levels in maize seedlings: Distribution and translocation in normal and cadmium-exposed plants. **Plant Physiol.,** 98: 128-138.

Rengel, Z. (1999). Heavy Metals as Essential Nutrients**.** In**: Heavy Metal Stress in Plants: From Molecules to Ecosystems**, Prasad, M.N.V. and J. Hagemeyer (Eds.). Springer, Berlin, Germany, 1137-1144.

Rivetta A, Negrini N, Cocucci M (1997) Involvement of Ca2+-calmodulin in Cd^{2+} toxicity during the early phases of radish (Raphanus sativus L.) seed germination. **Plant Cell Environ** 20: 600–608

Rocha, L. A., Rosa, I. L. & R. S. Rosa.(1998). Peixes recifais da costa da Paraíba, Brasil. **Revista Brasileira de Zoologia** 15, 553-566

Rojas, M. & Brewer, S. (2007). Effect of natural antioxidants on oxidative stability of cooked, refrigerated beef and pork. **J. Food Sci.** 72, S282-S288.

Sagi, M. & Fluhr, R. (2006). Production of reactive oxygen species by plant NADPH oxidases. **Plant Physiol.,** 141: 336–340.

Sahw, B.P.; Sahu, S.K. & Mishra, R.K. (2004). Heavy metal induced oxidative damage in terrestrial plants. In: Presad, M.N.V. (Ed.), Heavy Metal Stress in Plants: From **Biomolecules to Ecosystems,** second ed. Springer, Berlin, pp84–126.

Sakihama, Y., Yamasaki, H., (2002). Lipid peroxidation induced by phenolics in conjunction with aluminum ions. Biol. Plant. 45, 249–254.

Sandalio, L.M.; Dalurzo, H.C.; Gmez, M.; Romero-Puertas, M.C. & del Rı´o, L.A. (2001). Cadamium-induced changes in the growth and oxidative metabolism of pea plants. **J. Exp. Bot.** 52: 2115–2126.

Sasse, A.; Colindres, P.; Brewer, M.S. (2009). Effect of natural and synthetic antioxidants on oxidative stability of cooked, frozen pork patties. **J. Food Sci.** 74(1), S30-S35.

Schaffer S, Müller WE, Eckert GP, Tocotrienols (2005) : Constitutional effects in aging and disease.**J Nutr.** 135: 151-154.

Schmidt, W. (2003). Iron solutions: acquisition strategies and signaling pathways in plants. **Trends Plant Sci.** 8: 188–193.

Schröder, J.; Paulus, S.; Brenk, P.; Stahl, T. & Mersch-Sundermann, V. (2008). Antigenotoxic properties of Eruca sativa (rocket plant), erucin and erysolin in human hepatoma (HepG2) cells towards benzo(a)pyrene and their mode of action. **Food Chem. Toxicol;** 46:2415–2421.

Schu¨tzendu¨bel, A.; Schwanz, P.; Teichmann, T.; Gross, K.; Langenfeld-Heyser, R.; God bold, D.L. & Polle A. (2001). Cadmium-induced changes in antioxidative systems, hydrogen peroxide content, and differentiation in Scots pine roots. **Plant Physiol.** 127: 887–898.

Schützendübel, A. & Polle, A. (2002). Plant responses to abiotic stresses: heavy metalinduced oxidative stress and protection by mycorrhization. **J. Exp. Bot.** 53: 1351–1365.

Sebastianil.,Scebba F., Ton Getti R.(2004) Heavy metal accumulation and growth responses in poplar clones Eridano (Populus deltoides maximowiczii) and I-214 (P. euramericana) exposed to industrial waste. **Env. Exp. Bot**. 52, 79.

Seregin, I.V.; Kozhevnikova, A.D. (2006). Physiological role of nickel and its toxic effects on higher plants. **Russ. J. Plant Physiol.,** 53: 257–277.

Serrano-Martínez, A., Fortea, F.M., Del Amor, F.M. and Núñez-Delicado, E. (2008). Kinetic characterization and thermal inactivation study of partially purified red pepper (Capsicum annuum L.) peroxidase. **Food Chemistry**, 107: 193-199.

Seth C.S., Remans T., Keunen E., Jozefczak M., Gielen H., Opdenakker K., Weyens N., Vangronsveld J.(2012), Cuypers A. Phytoextraction of toxic metals: a central role for glutathione. **Plant Cell and Environment**. 35:334–346.

Shahidi, F. & Wanasundara, P.K. (1992). Phenolic antioxidants. Crit. Rev. **Food Sci. Nutr.** 32(1), 67-103.

Shanker, A.K.; Cervantes, C.; Loza-Tavera, H. & Avudainayagam, S. (2005). toxicity in plants. **Environ. Int.** 31: 739-753.

Shanker, A.K.; Sudhagar, R., Pathmanabhan, G. (2003a). **Growth Phytochelatin SH and antioxidative response of Sunflower as affected by chromium speciation. In:** 2nd international congress of plant physiology on sustainable plant productivity under changing environment, New Delhi.

Sharma, P. & Dubey, R.S (2005). Lead toxicity in plants. Braz. **J. Plant Physiol**., 17 (1), 35-52.

Sharma, P. & Dubey, R.S. (2007). Involvement of oxidative stress and role of antioxidative defense system in growing rice seedlings exposed to toxic concentrations of aluminum. **Plant Cell Rep.** 26: 2027-2038.

Sharma, S.S. & Dietz, K.-J. (2009). The relationship between metal toxicity and cellular redox imbalance. **Trends in Plant Science,** Vol.14, No.1, 43-50.

Shengwen Shen, Xing-Fang Li, William R. Cullen, Michael Weinfeld, and X (2013) . Chris Le Arsenic Binding to Proteins. **Chem Rev.** 113(10): 7769–7792.

Simsek, S. & A. Yemenicioglu. (2007). Partial purification and kinetic characterization of mushroom stem polyphenoloxidase and determination of its storage stability in different lyophilized forms. **Process Biochemistry,** 42 (6): 943-950.

Siroka, B.; Huttova, J.; Tamás, L.; Simonoviva, M. & Mistrik, I. (2004). Effect of cadmium on hydrolytic enzymes in maize root and coleoptile. **Biologia,** 59, 513-517.

Smith, K.S.; Balistrieri, L.S.; Smith, S.M & R. C. Severson. (1997). "**Distribution and Mobility of Molybdenum in the Terrestrial Environment,"** in Molybdenum in Agriculture, U.C. Gupta, Ed. Cambridge: Cambridge University Press, 23-46.

Stroinski, A. & M. Kozlowska. (1997). Cadmium-induced oxidative stress in potato tuber. **Acta Soc. Bot. Poloniae,** 66: 189-195.

Stroud, J.L.; Zhao, F.J.; Buchner, P.; Shinmachi, F.; McGrath, S.P.; Abecassis, J.; Hawkesford, M.J. & Shewry, P.R. (2010). Impacts of sulphur nutrition on selenium and molybdenum concentrations in wheat grain," **J. Cereal Sci.,** vol.52: 111-113.

Suzuki, K.; Yano, A. & Shinshi, H. (1999). Slow and prolonged activation of the p47 protein kinase during hypersensitive cell death in cultured tobacco cells. **Plant Physiol.** 119, 1465-1472.

Sweetlove, L.J. & Foyer, C.H, (2004) Roles for reactive oxygen species and antioxidants in plant mitochondria. In DA Day, AH Millar, **J Whelan,** eds, Plant Mitochondria: From Genome to Function, Vol 1, Advances in Photosynthesis and Respiration. Kluwer Academic Press, Dordrecht, The Netherlands, pp 307–320.

Valko M, Morris H and Cronin MTD (2005). Metals, Toxicity and Oxidative Stress. **Current Medicinal Chemistry**, 12: 1161-1208.

Van Assche, F. & Clijsters, H. (1986). Inhibition of photosynthesis in Phaseolus vulgaris by treatment with toxic concentration of zinc: Effects on electron transport and photophosphorylation. **J. Plant Physiol**., 66: 717-721

Van Assche, F. & Clijsters, H. (1990). Effect of metals on enzyme activity in plants. **Plant Cell Environ**. 13: 195–206.

Voet & Voet.(2011). **Biochemistry text chapter on Rate of Enzymatic Reactions**. pp. 482-500. Fourth edition. USA: Ed. John Wiley & Sons, Inc.

Vranova, E.; Inze, D. & Van Breusegem, F. (2002). Signal transduction during oxidative stress. **Journal of Experimental Botany**, Vol.53, No.372, 1227-1236.

Wang, C.Q. & Song, H. (2009). Calcium protects Trifolium repens L. seedlings against cadmium stress. **Plant Cell Rep.,** 28: 1341-1349.

Whitaker, J.R. (1996). **Enzymes. In Food Chemistry,** O.R. Fennema (Eds), Marcel Dekker Inc., New York, NY. p. 431–530,

WHO. (1988). Environmental health criteria, Chromium. WHO, Geneva. Panda SK, Patra HK. Physiology of chromium toxicity in plants **A review Plant Physiol Biochem.,** 24: 10-17.

Willcox, J.K.; Ash, S.L. & Catignani, G.L. (2004). Antioxidants and prevention of chronic disease. Review. Crit. Rev. **Food Science Nutrition.** 44:275–295.

Wo, J.E.P. (1997). Oxidative burst: an early plant response to pathogen infection**. Biochem. J.** 322, 681, 1997**.**

Wong P.Y.Y., Kitts D.D., 2006. Studies on the dual antioxidant and actibacterial properties of parsley (Petroselium crispum) and cilantro (Coriandrum sativum) extracts. **Food Chem.** 97,505-515.

Xiong, Y.L.; Decker, E.A. & Robe, G.H. (1993). Gelation of crude myofibrillar protein isolated from beef heart under antioxidative conditions. **J. Food Sci.** 58(6), 1241-1244.

Xu, F.J.; Li, G.; Jin, C.W.; Liu, W.J.; Zhang, S.S. & Zhang, Y.S.; et al (2012). Aluminum induced changes in reactive oxygen species accumulation, lipid peroxidation and antioxidant capacity in wheat root tips. **Biol. Plant.** 56: 89-96.

Ya, M.H.; Sakiha, M.Y. & Ik, E.N. (1997). Flavonoidperoxidase reaction as a detoxification mechanism of plant cell against H_2O_2. **Plant Physiol**. 115, 1405.

Yan, R.; Gao, S.; Yang, W.; Cao, M.; Wang, S. & Chen, F. (2008): Nickel toxicity induced antioxidant enzyme and phenylalanine ammonia-lyase activities in Jatropha curcas L. cotyledons. **Plant Soil Environ**, 54: 294–300.

Yaniv, Z.; Schafferman, D. & Amar, Z. (1998). Tradition, uses and biodiversity of rocket (Eruca sativa, Brassicaceae) in Israel. **Econ. Bot.,** 52(4): 394–400.

Yoruk, R. & Marshall, M.R. (2003). Physicochemical properties and function of plant polyphenol oxidase: a review. **J. Food Biochem.** 27: 361–422.

Young, I. & Woodside, J. (2001). Antioxidants in health and disease. **Journal of Clinical Pathololology**. 54: 176–186

Yruela, I. (2005). Copper in plants. Braz. **J. Plant Physiol**., 17: 145–156.

Zayed, C. M. Lytle, J. H. Qian, and N. Terry.(1998) “Chromium accumulation, translocation and chemical speciation in vegetable crops,” **Planta**, vol. 206, no. 2, pp. 293–299.

Zenk, M.H. (1996). Heavy metal detoxification in higher plants: a review. Gene, 179: 21–30.

Zheng, W. & Wang, S.Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. **J Agric Food Chem**., 49:5165-5170.

Zornoza, P.; Vazquez, S.; Esteban, E. & Fern, A.M. (2002). CARP ENA R. Cadmium-stress in nodulated white lupin: strategies to avoid toxicity. **Plant Physiol. Biochem.** 40: 1003

Appendix (I)

Standard curve for protein determination by Lowry method

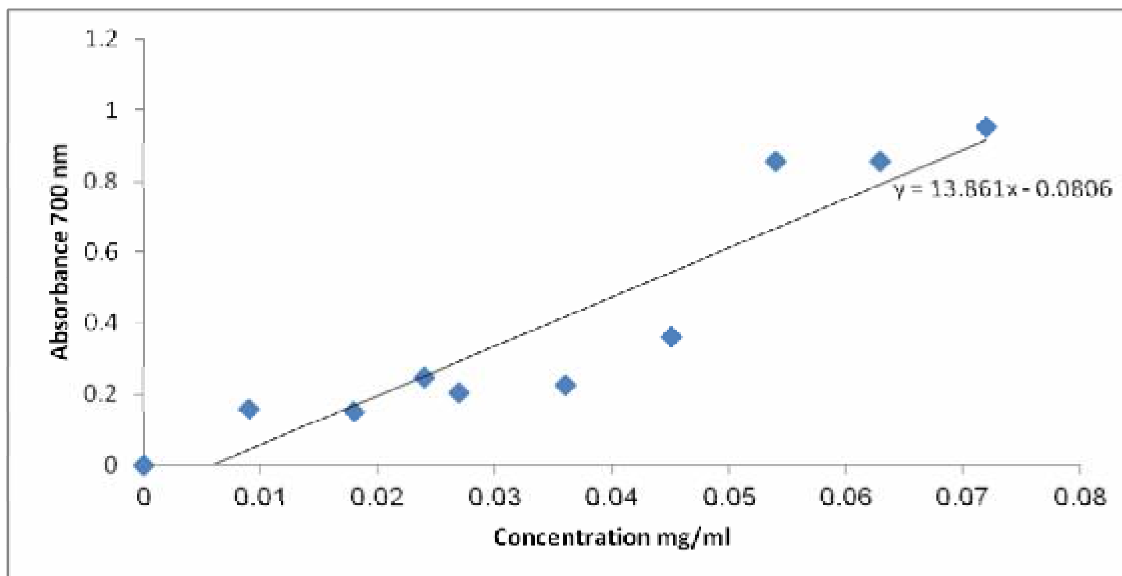


Figure (1)
Determination of protein content in *Rosmarinus officinalis*.

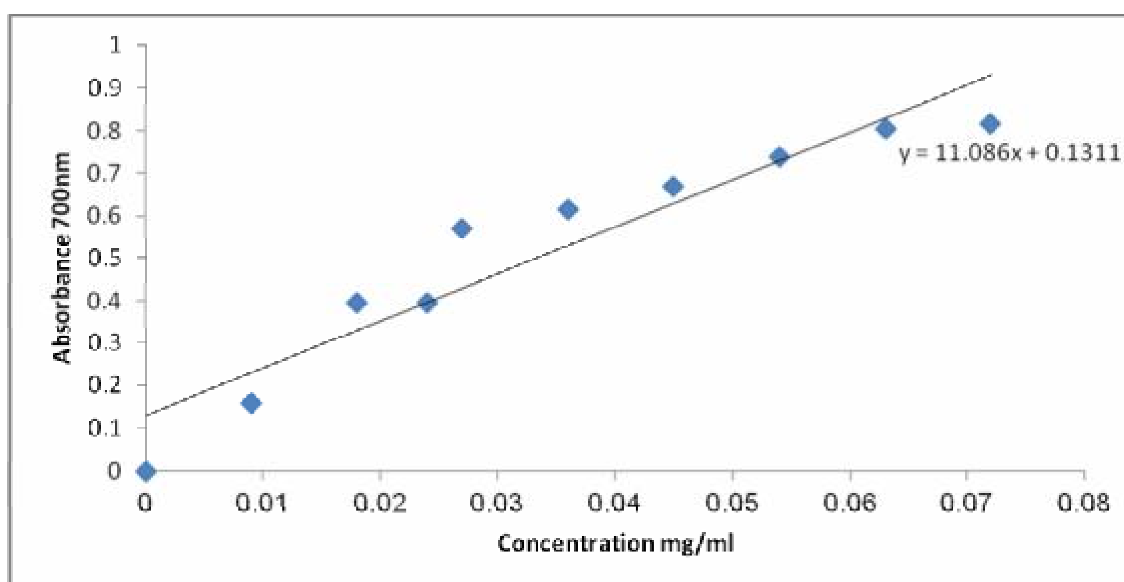


Figure (2)
Determination of protein content in *Petroselium cripsum*.

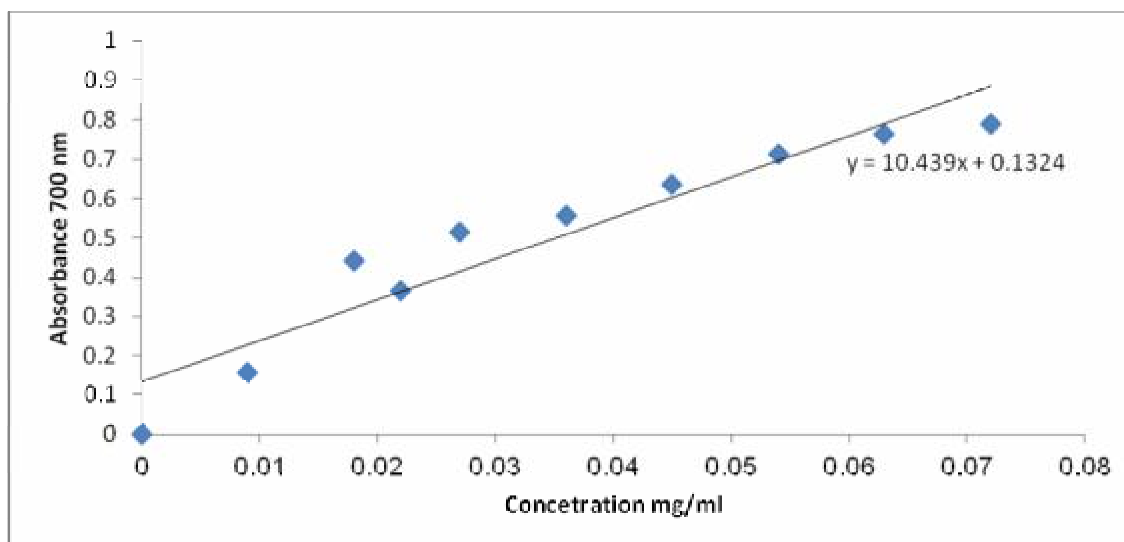


Figure (3)
Determination of protein content in *Eruca Sativa*.

Appendix (II)

Lineweaver-Burk double reciprocal plots.

A- Uncompetitive inhibition on:

1- Crude enzyme (POX) extracts from rosemary, parsley and rocket.

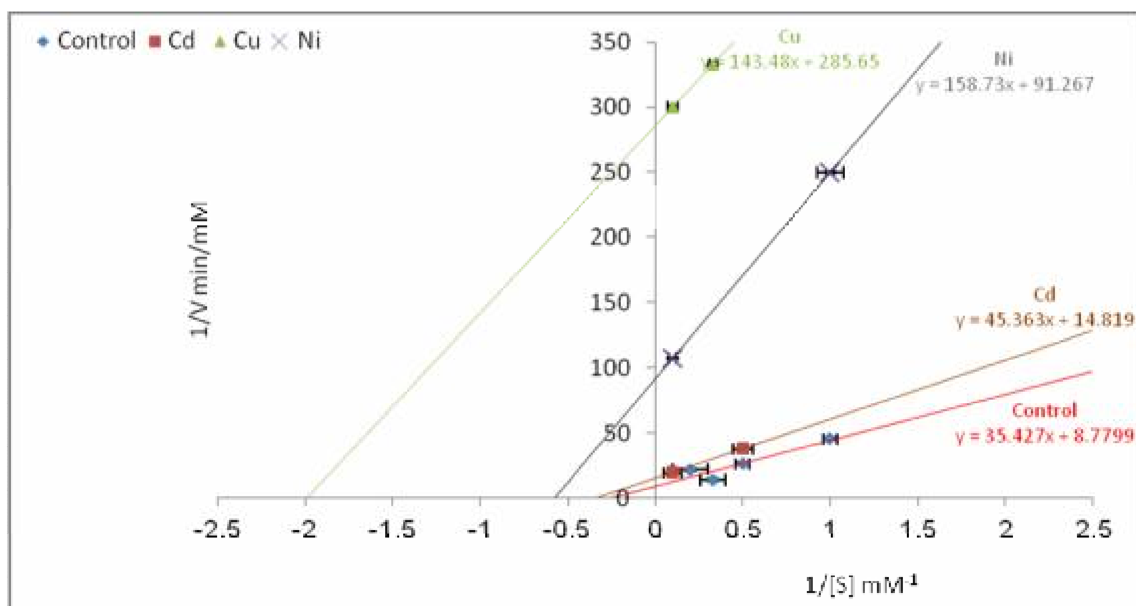


Figure (4)

etermination of K_m and V_{max} values for crude enzyme (POX) extracts of *Rosmarinus oficinalis* in control and in presence of Cd^{2+}, Cu^{2+} and Ni^{2+} at 400µM. Mean ±SD (n=3).

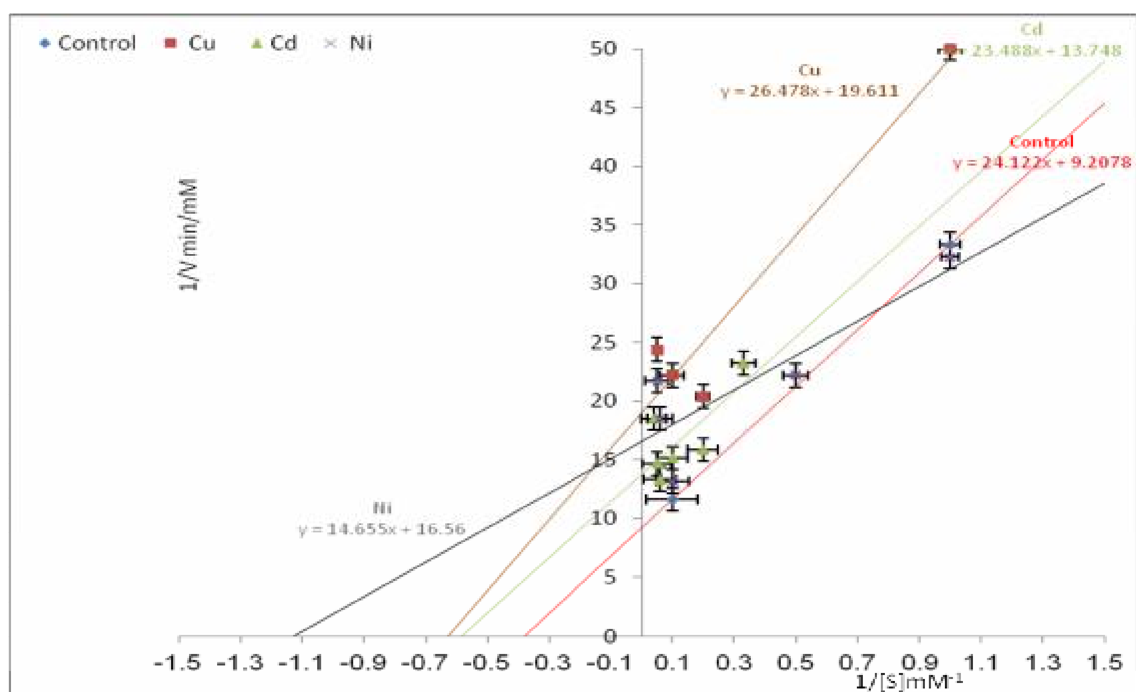


Figure (5)

Determination of K_m and V_{max} values for crude enzyme (POX) extracts of *Petroselium cripsum* in control and in presence of Cd^{2+}, Cu^{2+} and Ni^{2+} at 400µM. Mean ±SD (n=3).

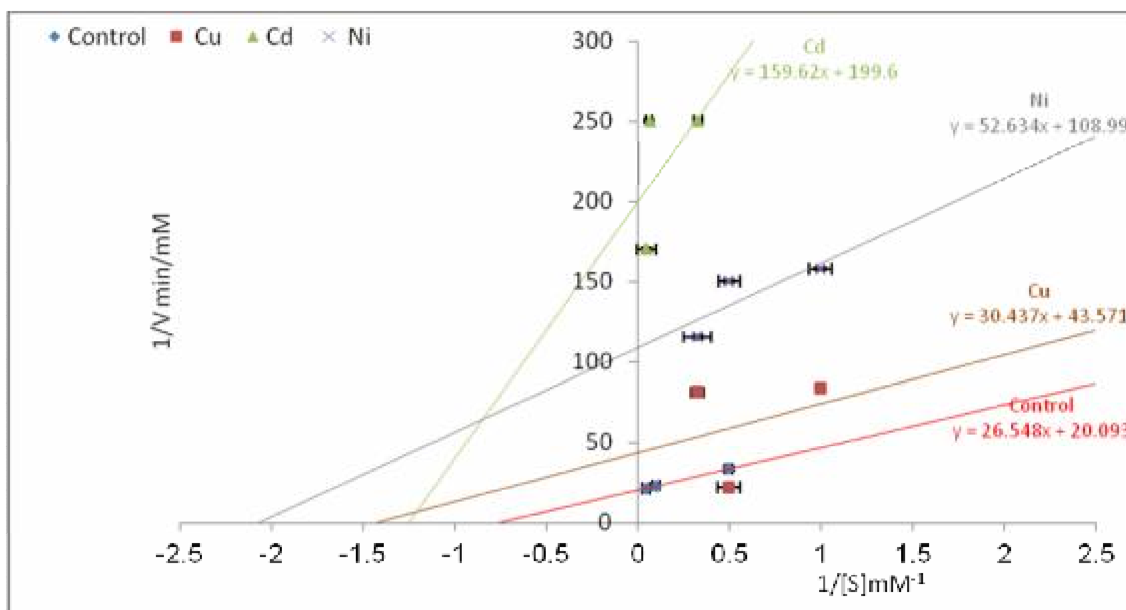


Figure (6)
Determination of K_m and V_{max} values for crude enzyme (POX) extracts of *Eruca Sativa* in control and in presence of Cu^{2+}, Cd^{2+} and Ni^{2+} at 400µM. Mean ±SD (n=3).

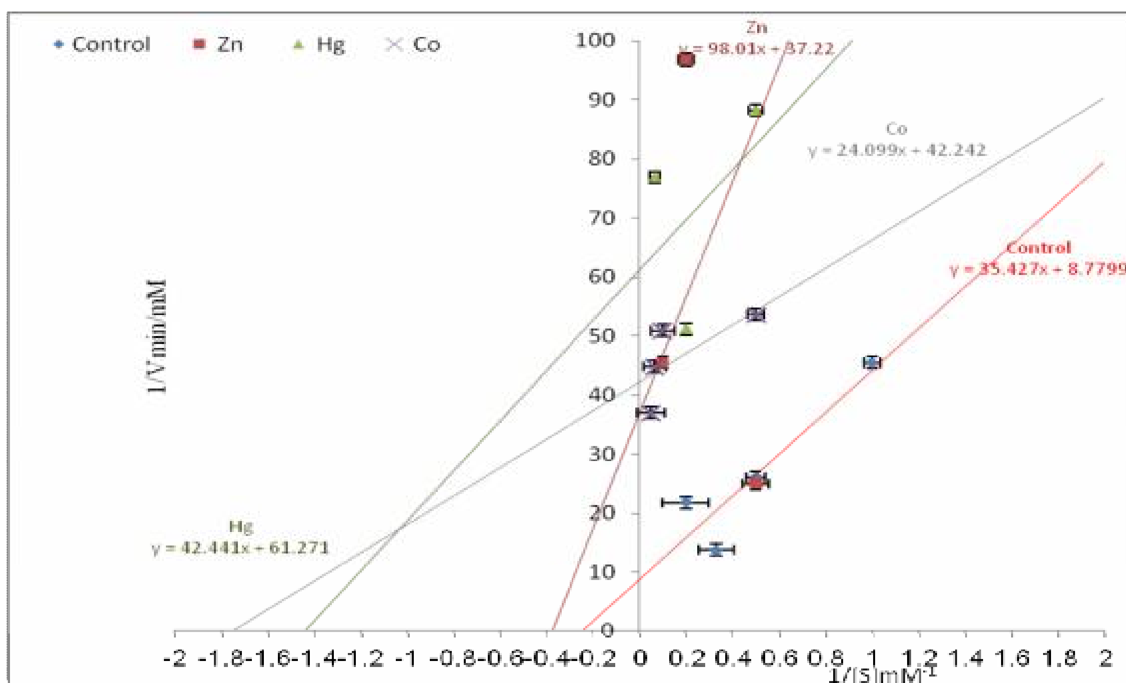


Figure (7)
Determination of K_m and V_{max} values for crude enzyme (POX) extracts of *Rosmarinus oficinalis* in control and in presence of Zn^{2+}, Hg^{2+} and Co^{2+} at 400µM. Mean ±SD (n=3).

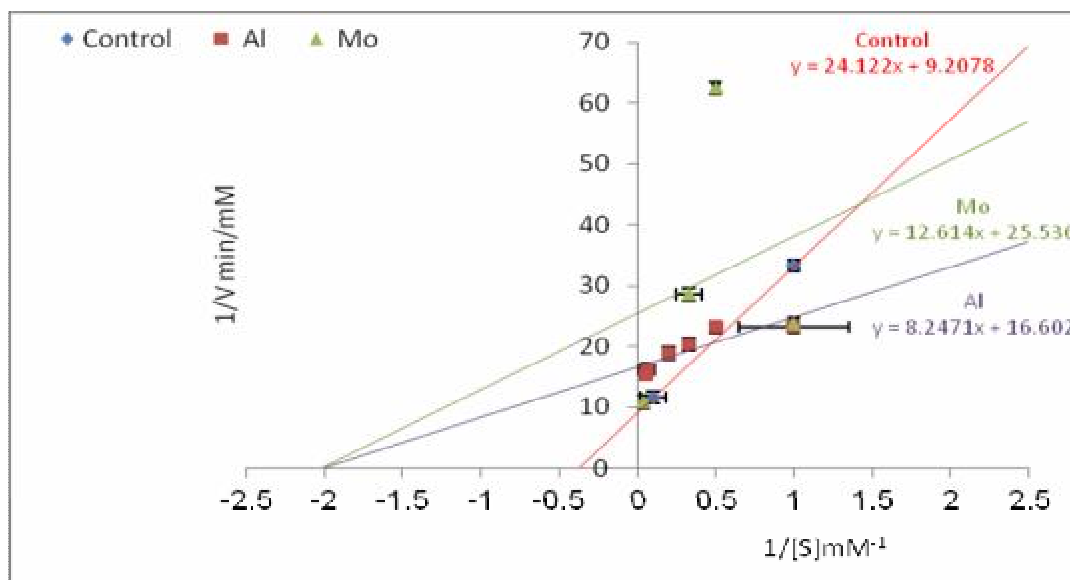


Figure (8)
Determination of K_m and V_{max} values for crude enzyme (POX) extracts of *Petroselium cripsum* in control and in presence of Mo^{2+} and Al^{3+} at 400µM. Mean ±SD (n=3).

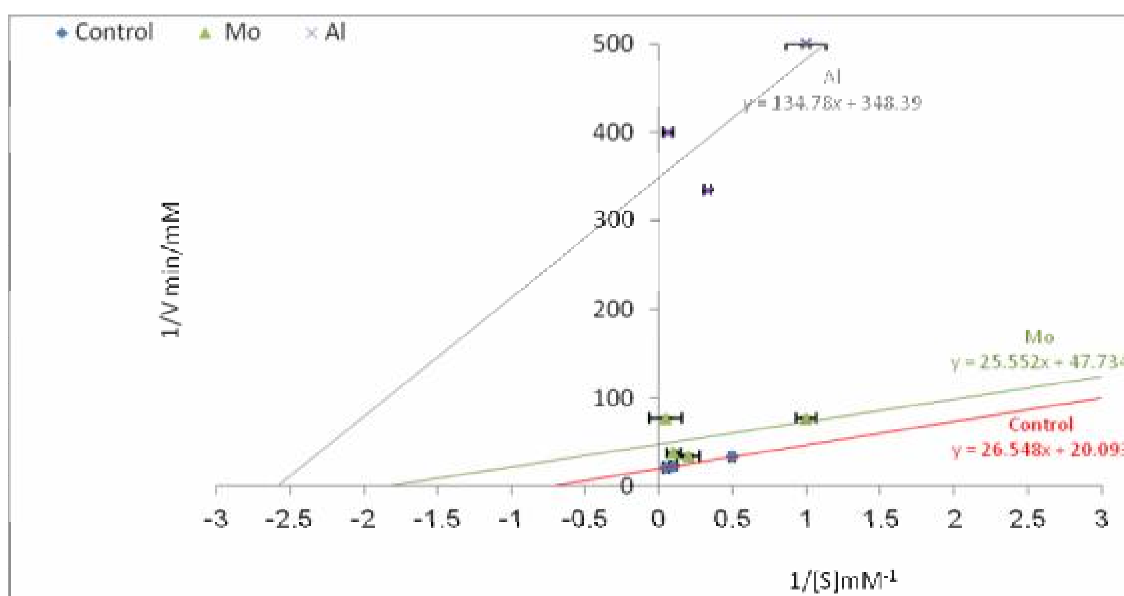


Figure (9)
Determination of K_m and V_{max} values for crude enzyme (POX) extracts of *Eruca Sativa* in control and in presence of Cr^{2+}, Mo^{2+} and Al^{3+} at 400µM. Mean ±SD (n=3).

2- Crude enzyme (PPO) extracts from rosemary, parsley and rocket.

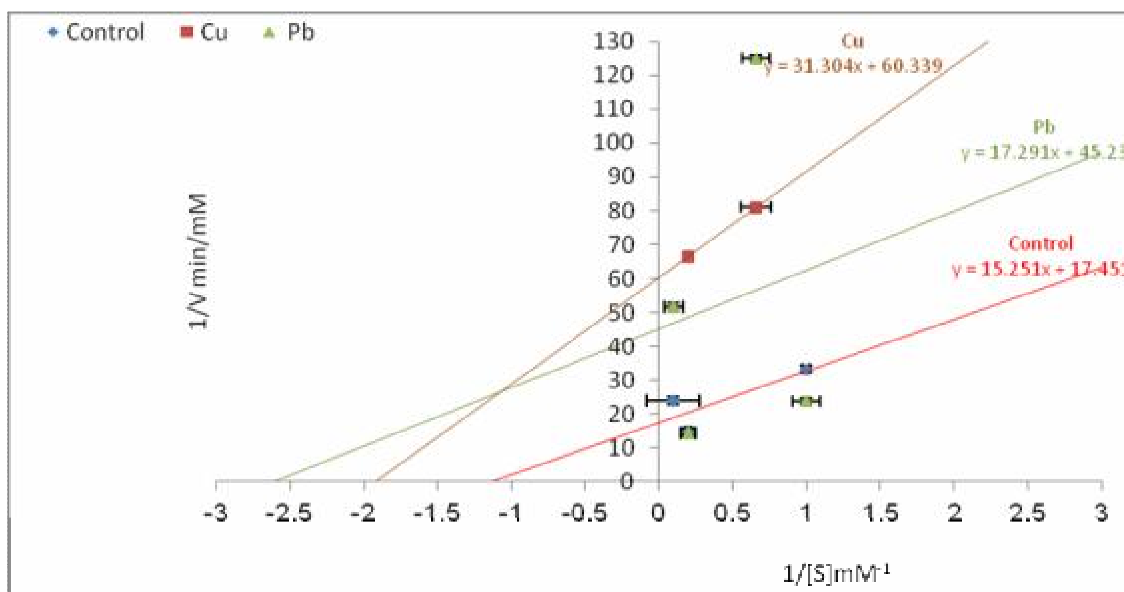


Figure (10)
Determination of K_m and V_{max} values for crude enzyme (PPO) extracts of *Rosmarinus oficinalis* in control and in presence of Cu^{2+} and Pb^{2+} at 400µM. Mean ±SD (n=3).

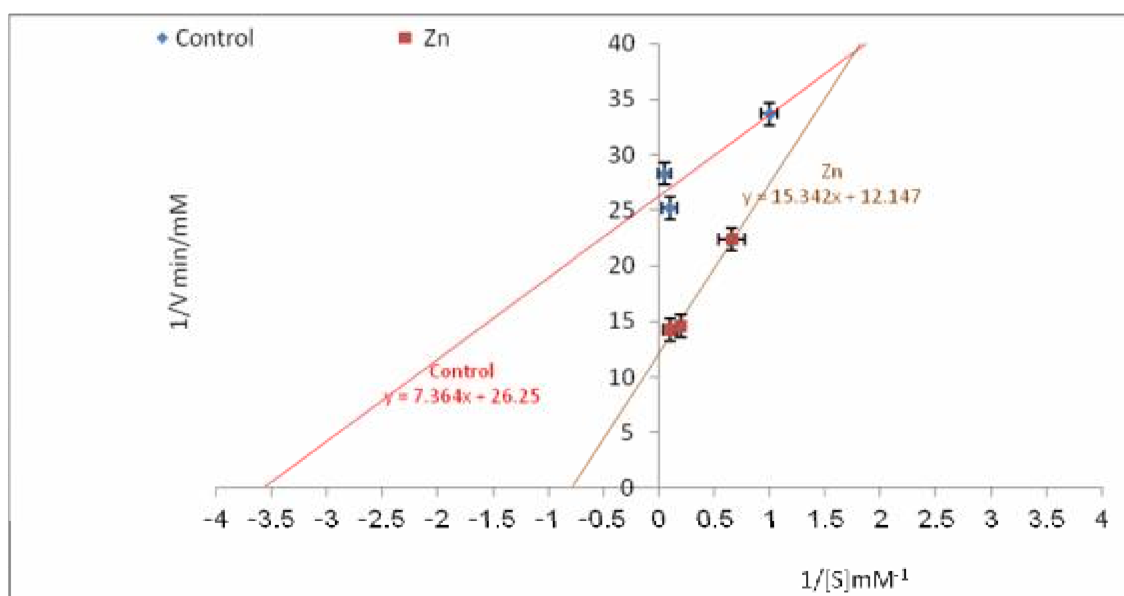


Figure (11)
Determination of K_m and V_{max} values for crude enzyme (PPO) extracts of *Petroselium cripsum* in control and in presence of Zn^{2+} at 400µM. Mean ±SD (n=3).

B- Noncompetitive inhibition on:

1- Crude enzyme (POX) extracts from rosemary, parsley and rocket.

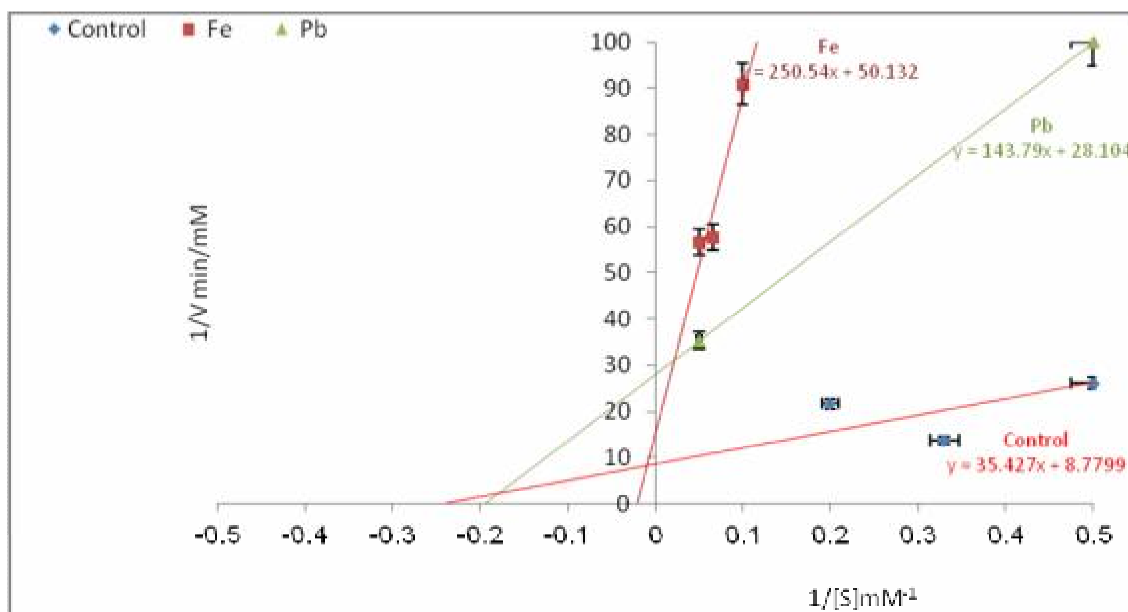


Figure (12)
Determination of K_m and V_{max} values for crude enzyme (POX) extracts of *Rosmarinus oficinalis* in control and in presence of Fe^{3+} and Pb^{2+} at 400µM. Mean ±SD (n=3).

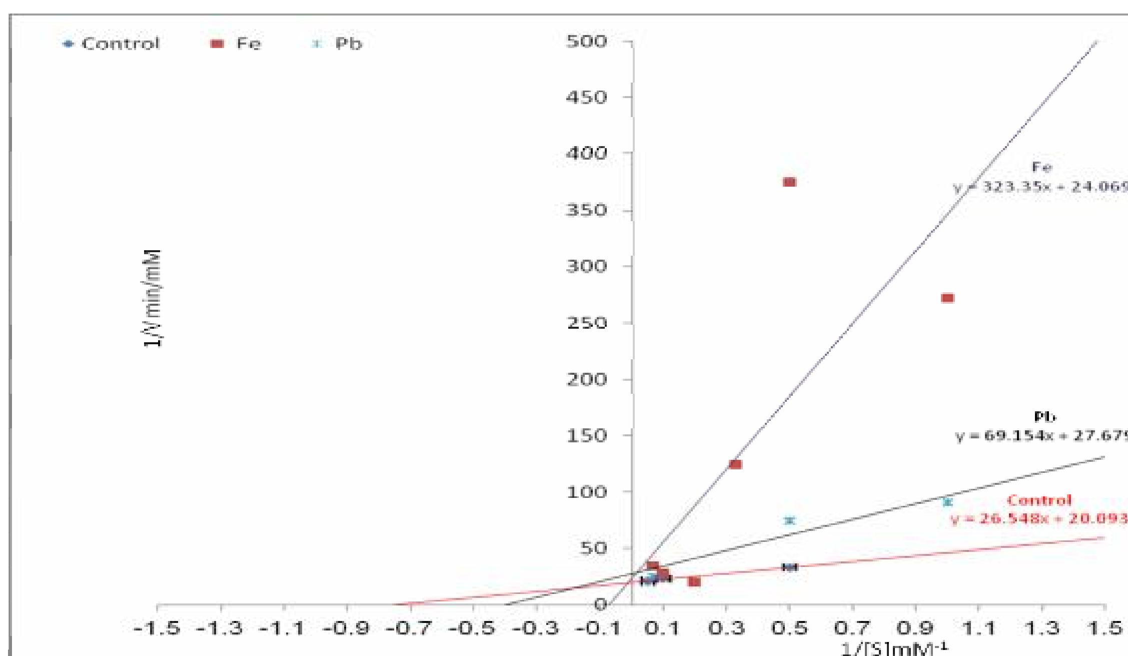


Figure (13)
Determination of K_m and V_{max} values for crude enzyme (POX) extracts of *Eruca Sativa* in control and in presence of Fe^{3+} and Pb^{2+} at 400µM. Mean ±SD (n=3).

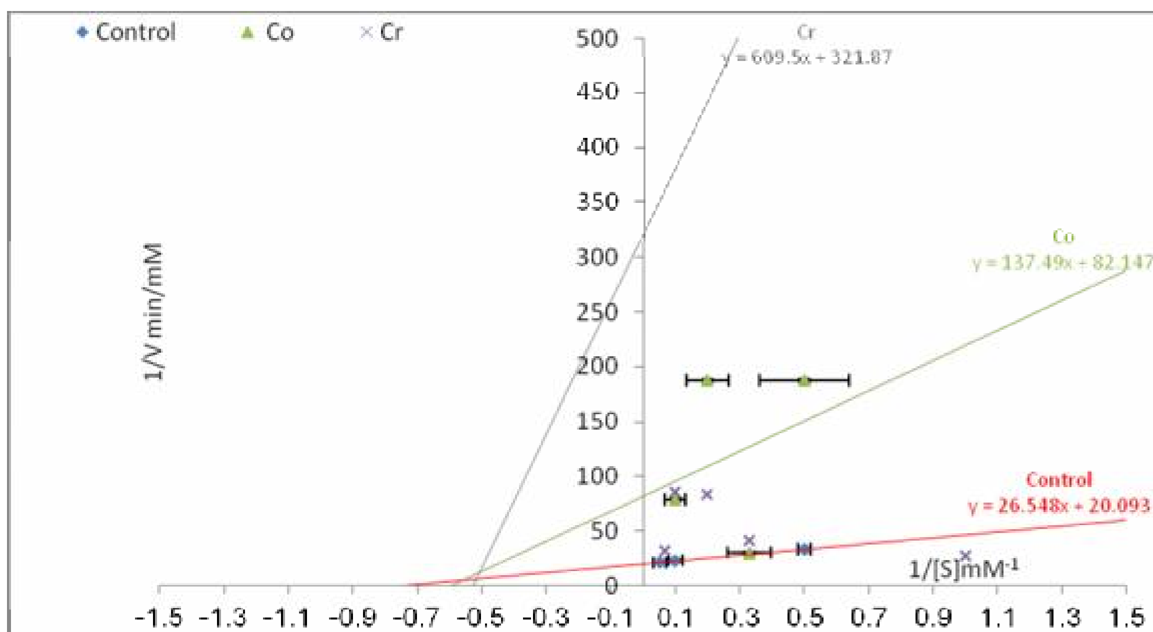


Figure (14)

Determination of K_m and V_{max} values for crude enzyme (POX) extracts of *Eruca Sativa* in control and in presence of Cr^{2+} and Co^{2+} at 400µM. Mean ±SD (n=3).

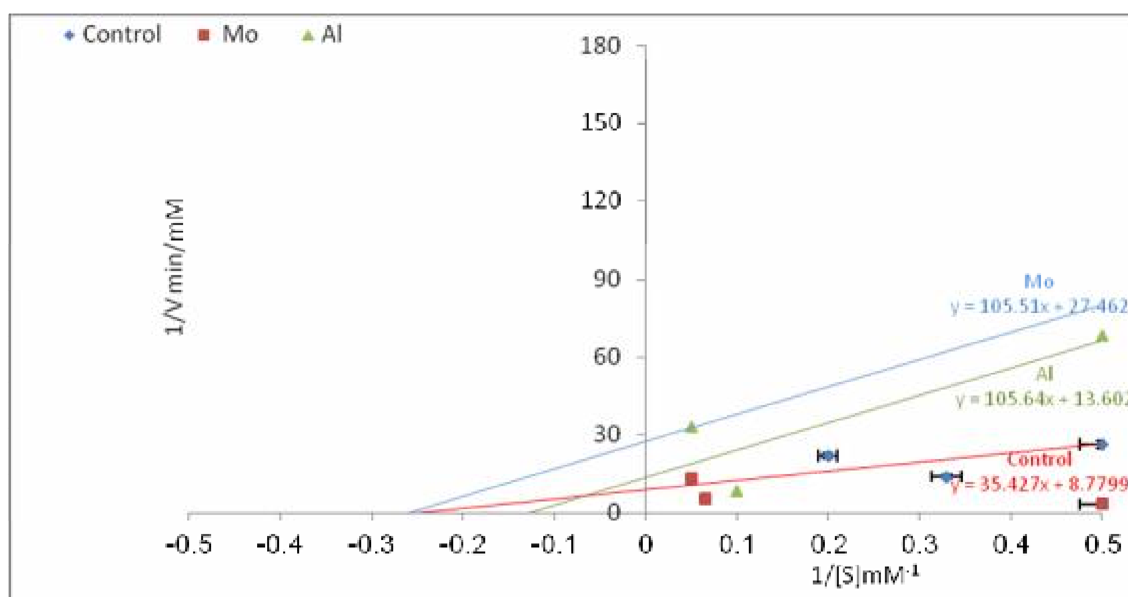


Figure (15)

Determination of K_m and V_{max} values for crude enzyme (POX) extracts of *Rosmarinus oficinalis* in control and in presence of Mo^{2+} and Al^{3+} at 400µM. Mean ±SD (n=3).

2- Crude enzyme (PPO) extracts from rosemary, parsley and rocket.

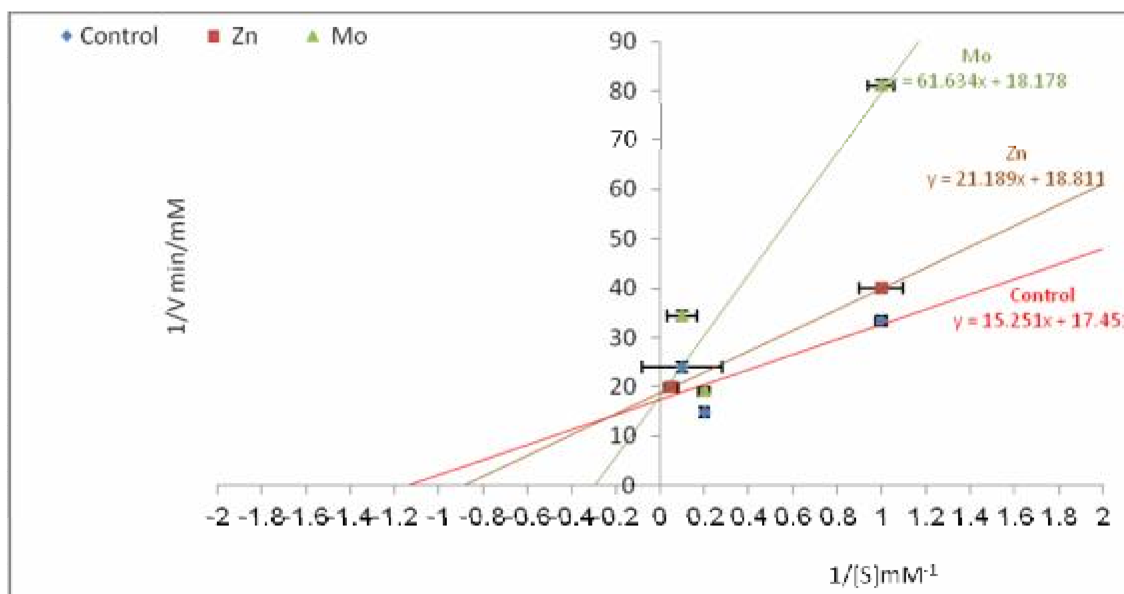


Figure (16)
Determination K_m and V_{max} values for crude enzyme (PPO) extracts of *Rosmarinus oficinalis* in control and in presence of Zn^{2+} and Mo^{2+} at 400µM. Mean ±SD (n=3).

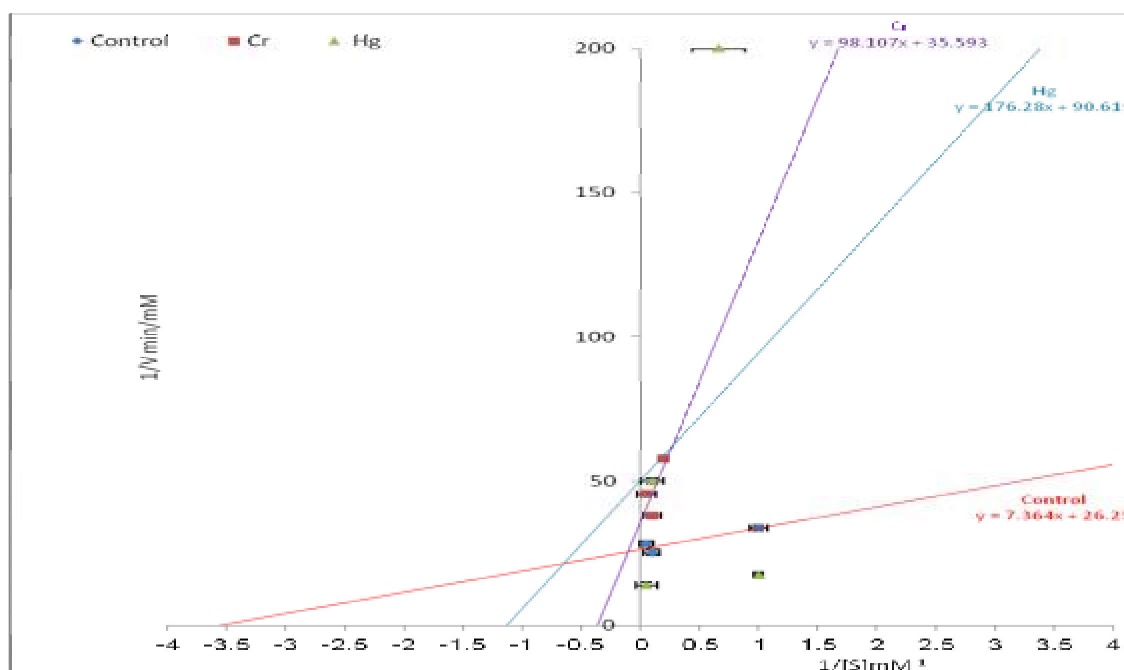


Figure (17)
K_m and V_{max} values for crude enzyme (PPO) extracts of *Petroselium cripsum* in control and in presence of Cr^{2+} and Hg^{2+} at 400µM. Mean ±SD (n=3).

C- Activation on:
1- Crude enzyme (POX) extracts from rosemary, parsley and rocket.

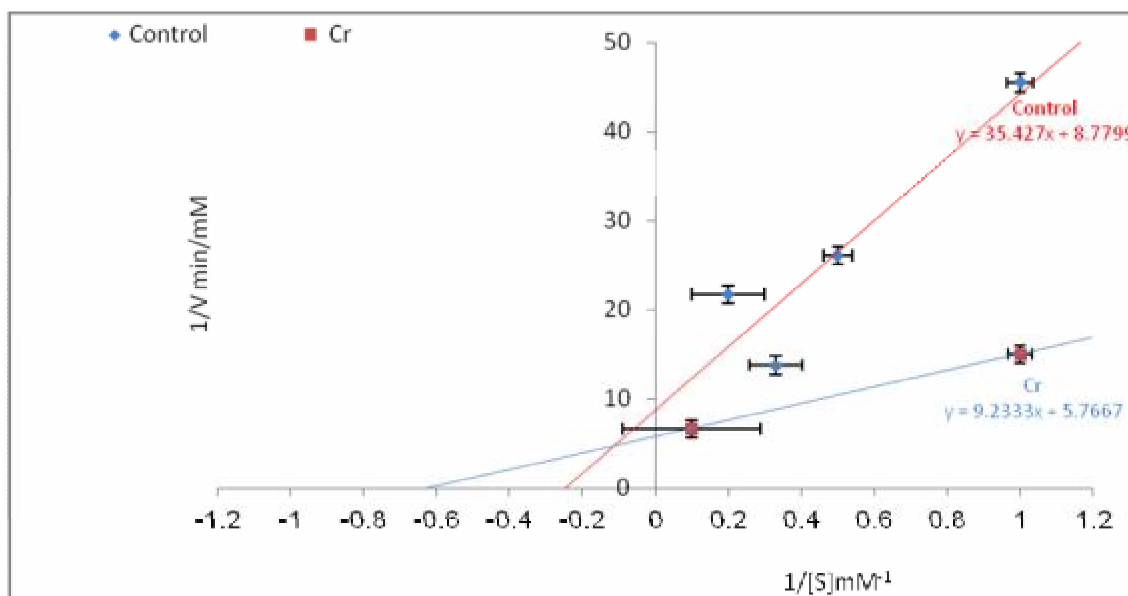


Figure (18)
Determination of K_m and V_{max} values for crude enzyme (POX) extracts of *Rosmarinus oficinalis* in control and in presence of Cr^{2+} at 400µM. Mean ±SD (n=3).

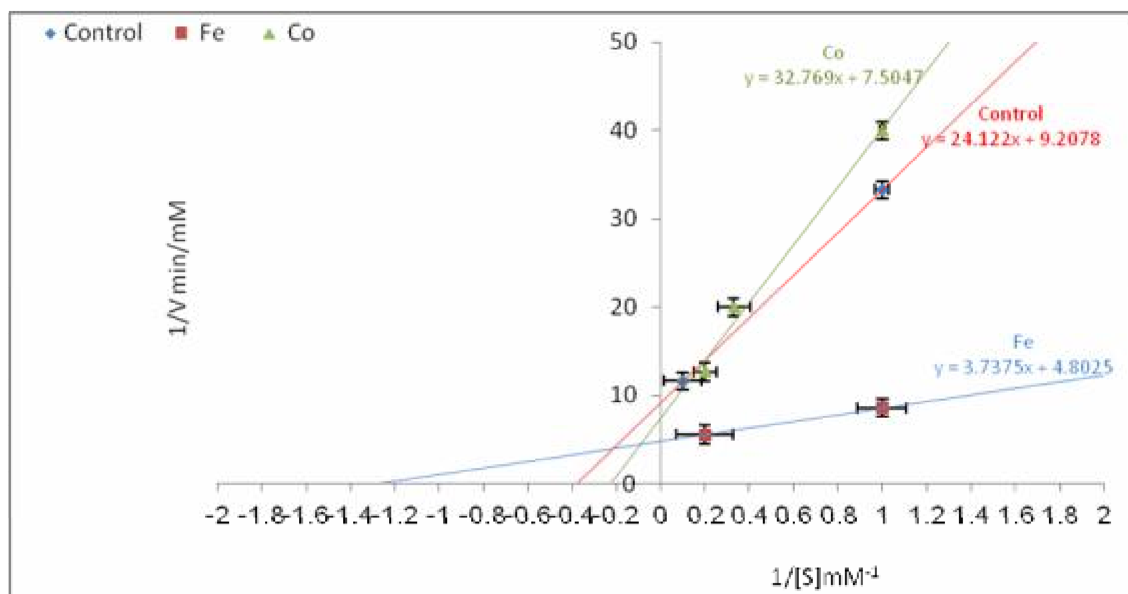


Figure (19)
Determination of K_m and V_{max} values for crude enzyme (POX) extracts of *Petroselium cripsum* in control and in presence of Fe^{3+} and Co^{2+} at 400µM. Mean ±SD (n=3).

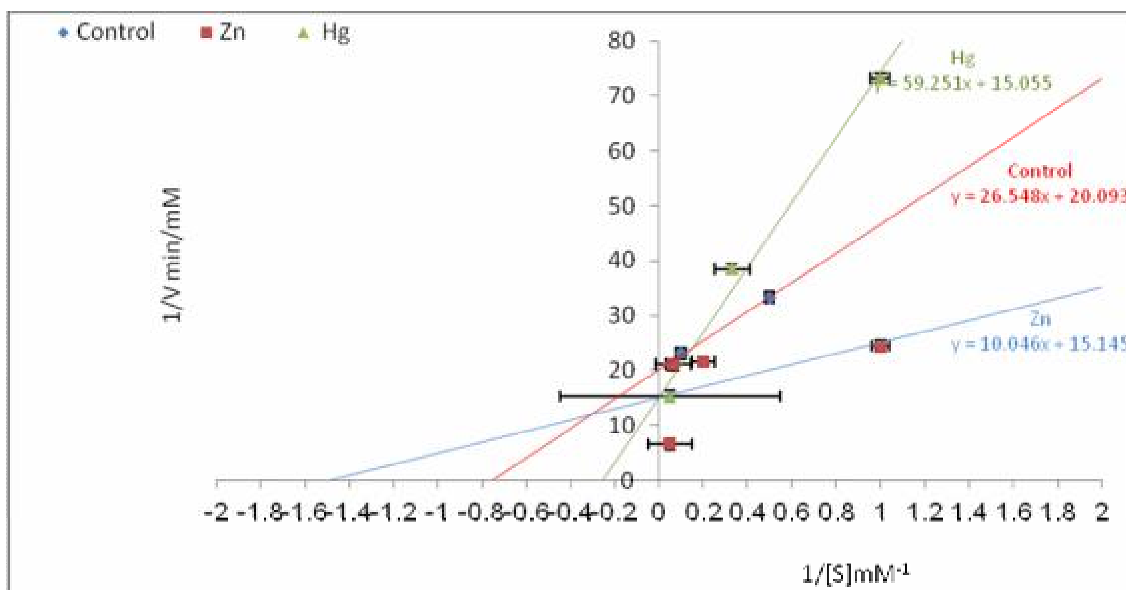


Figure (20)
Determination of K_m and V_{max} values for crude enzyme (POX) extracts of *Eruca Sativa* in control and in presence of Zn^{2+} and Hg^{2+} at 400µM. Mean ±SD (n=3).

2- Crude enzyme (PPO) extracts from rosemary, parsley and rocket.

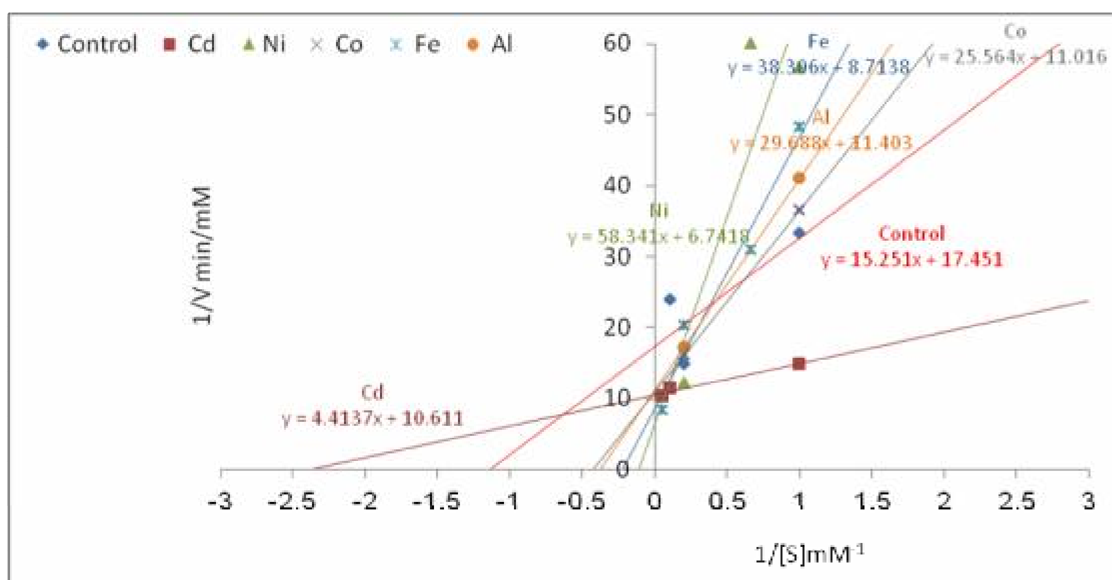


Figure (21)
Determination of Km and Vmax values for crude enzyme (PPO) extracts of *Rosmarinus oficinalis* in control and in presence of Cd2+, Ni2+, Fe3+, Al 3+ and Co2+ at 400µ. Mean ±SD (n=3).

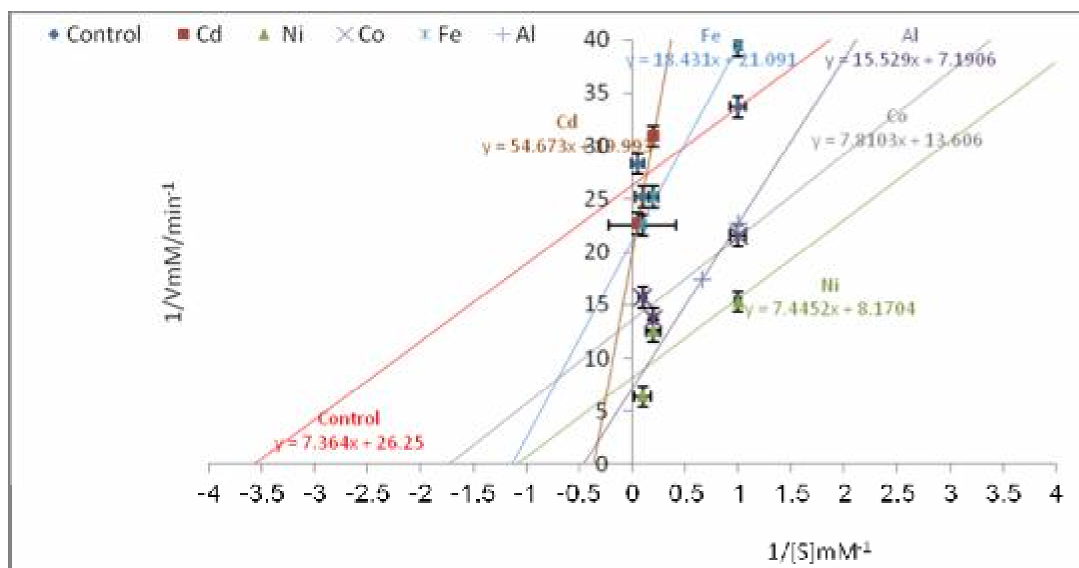


Figure (22)
Determination of Km and Vmax values for crude enzyme (PPO) extracts of *Petroselium cripsum* in control and in presence of of Cd^{2+}, Ni^{2+}, Fe^{3+}, Al^{3+} and Co^{2+} and Co2 at 400µM. Mean ±SD (n=3).

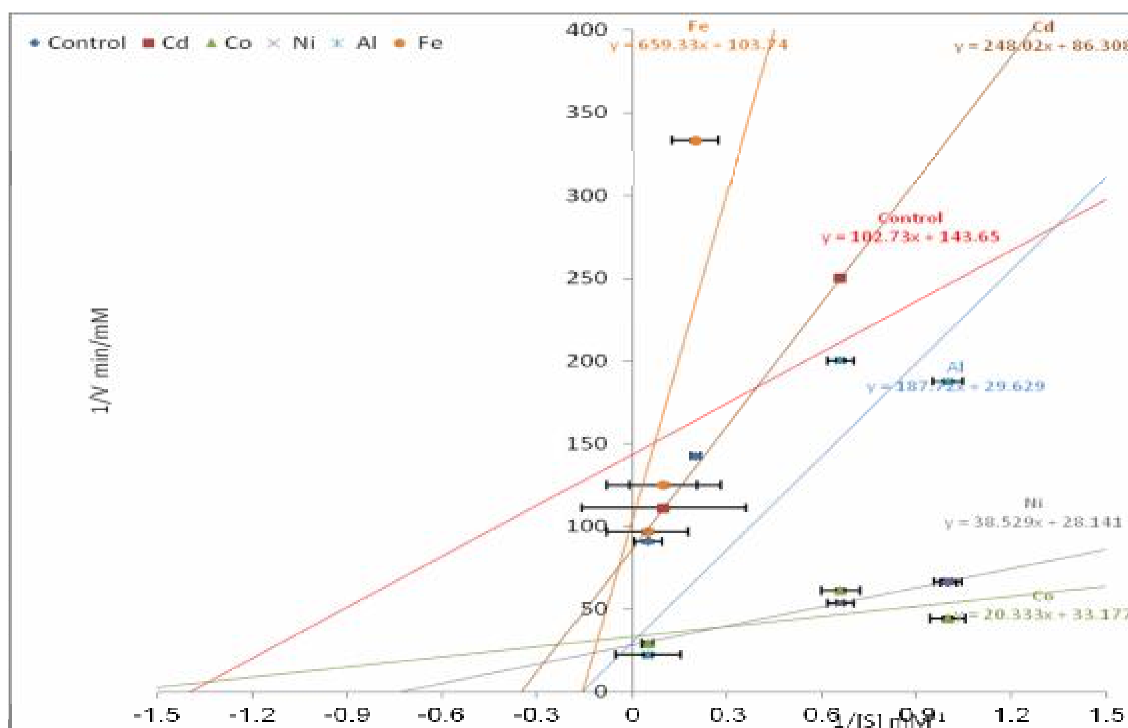


Figure (23)
Determination of K_m and V_{max} values for crude enzyme (PPO) extracts of *Eruca Sativa* in control and in presence of Cd^{2+}, Ni^{2+}, Fe^{3+}, Al^{3+} and Co^{2+} at 400µM. Mean ±SD (n=3).

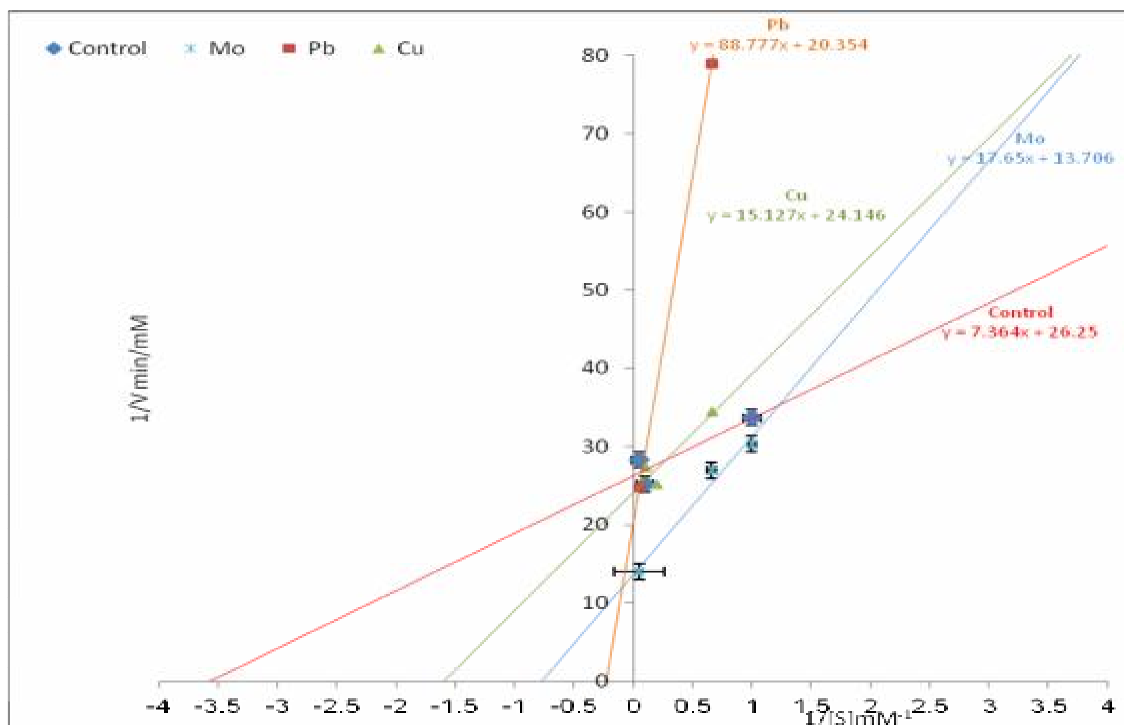


Figure (24)
Determination of K_m and V_{max} values for crude enzyme (PPO) extracts of *Petroselium cripsum* in control and in presence of Cu^{2+}, Mo^{2+} and Pb^{2+} at 400µM. Mean ±SD (n=3).

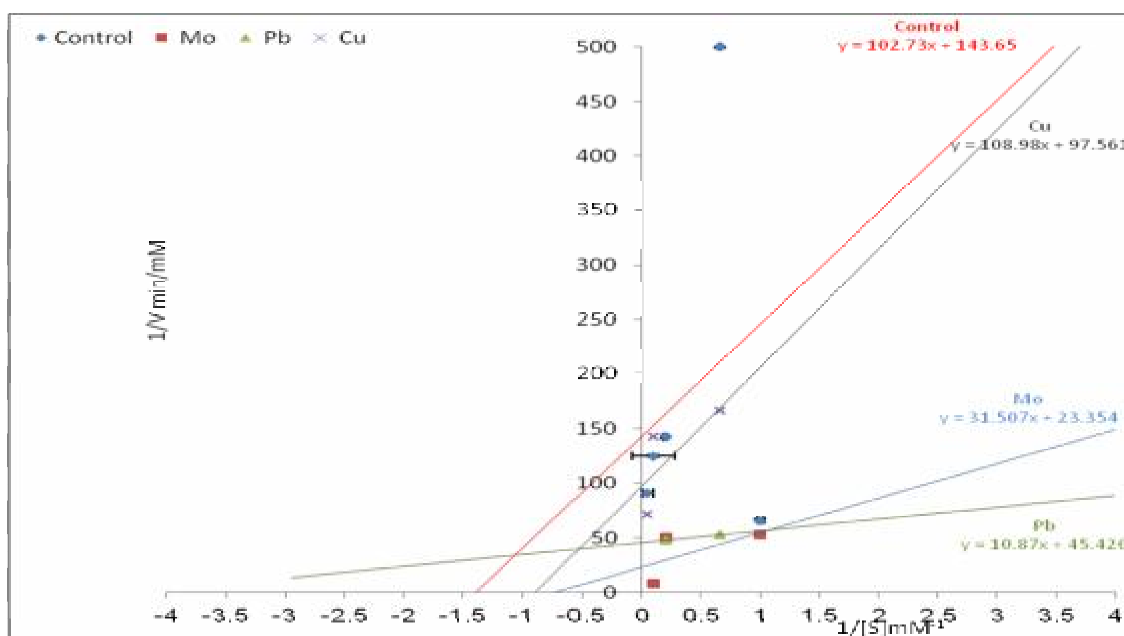


Figure (25)
Determination of K_m and V_{max} values for crude enzyme (PPO) extracts of *Eruca Sativa* in control and in presence of Cu^{2+}, Mo^{2+} and Pb^{2+} at 400µM. Mean ±SD (n=3).

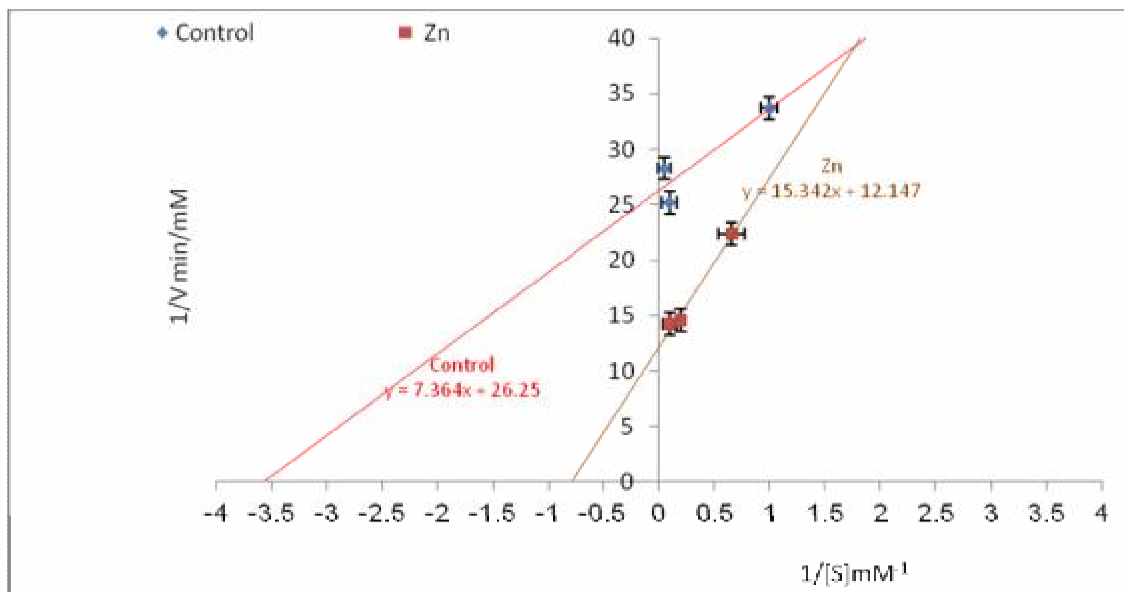


Figure (26)
Determination of K_m and V_{max} values for crude enzyme (PPO) extracts of *Petroselium cripsum* in control and in presence of Zn^{2+} at 400µM. Mean ±SD (n=3).

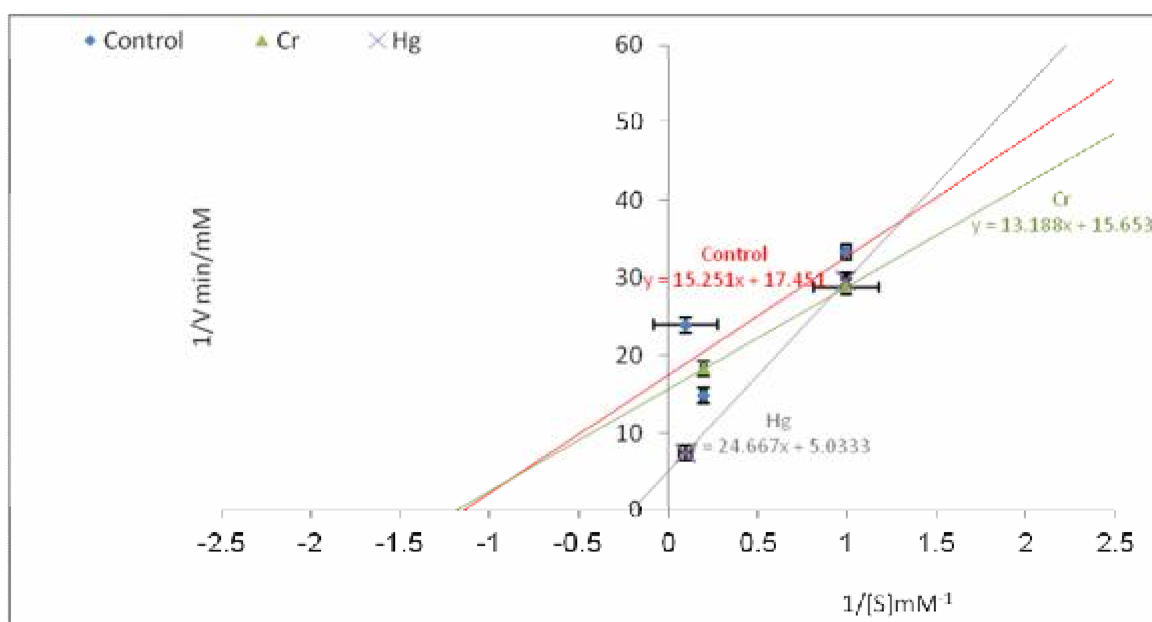


Figure (27)
Determination of K_m and V_{max} values for crude enzyme (PPO) extracts of *Rosmarinus oficinalis* in control and in presence of Hg^{2+} and Cr^{2+} at 400µM. Mean ±SD (n=3).

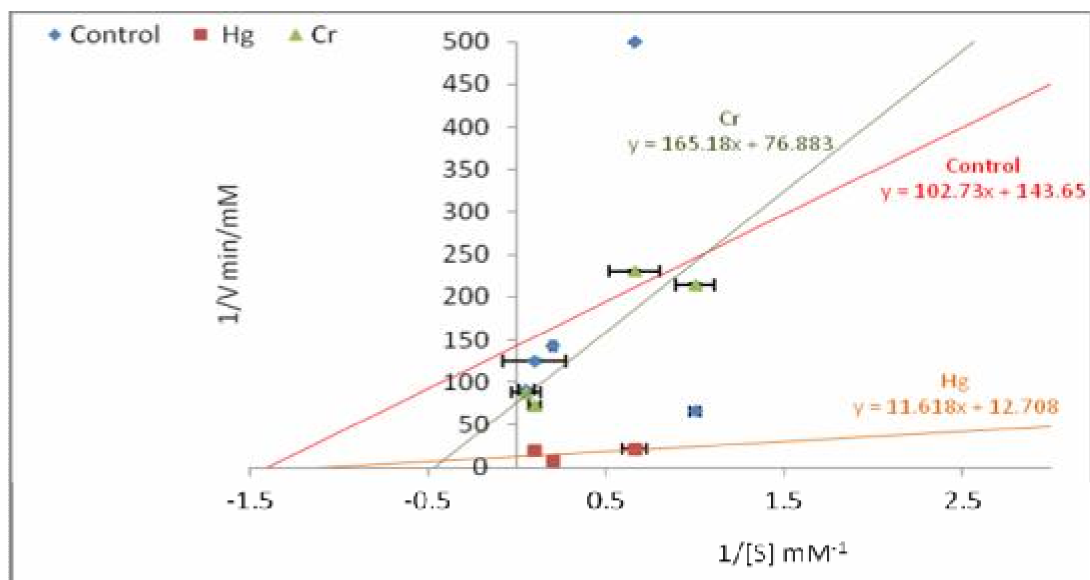


Figure (28)

Determination of K_m and V_{max} values for crude enzyme (PPO) extracts of *Eruca Sativa* in control and in presence of Hg^{2+} and Cr^{2+} at 400µM. Mean ±SD (n=3).

Appendix (III)

K*i* values for crude enzyme extract of rosemary, parsley and rocket.

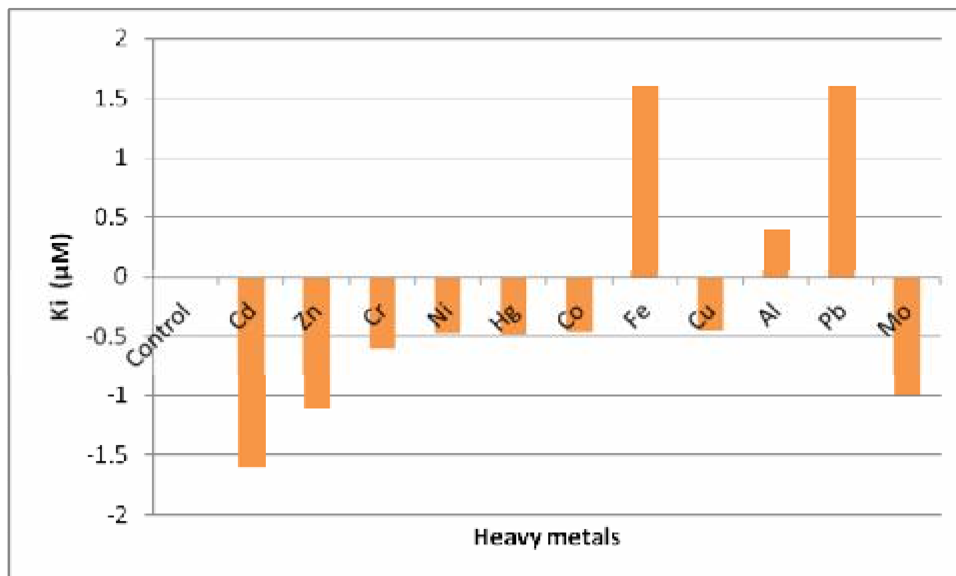


Figure (29)
K_i values for crude enzyme (POX) extracts of *Rosmarinus oficinalis* in control and in presence of different heavy metals at 400µM. Mean ±SD (n=3).

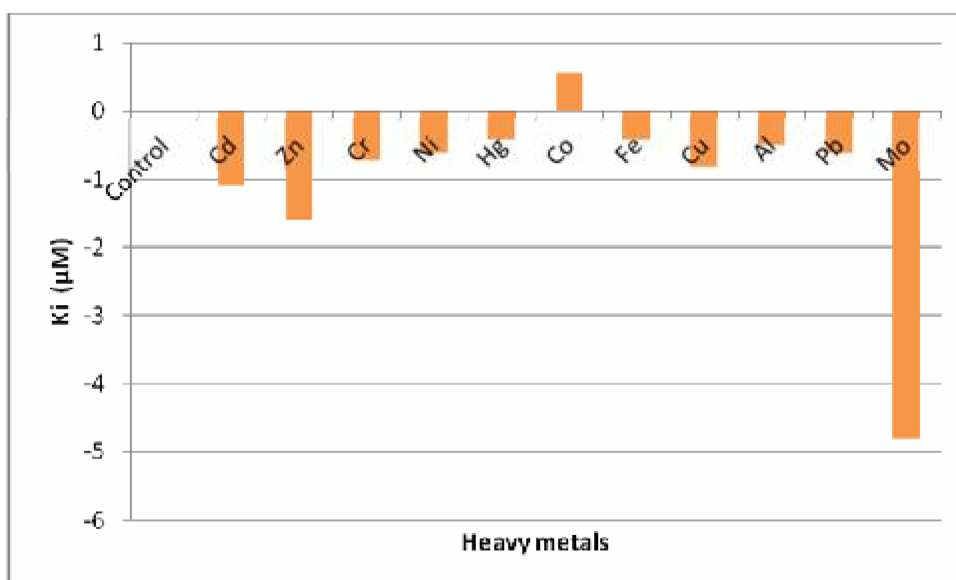


Figure (30)
K_i values for crude enzyme (POX) extracts of *Petroselium cripsum* in control and in presence of different heavy metals at 400µM. Mean ±SD (n=3).

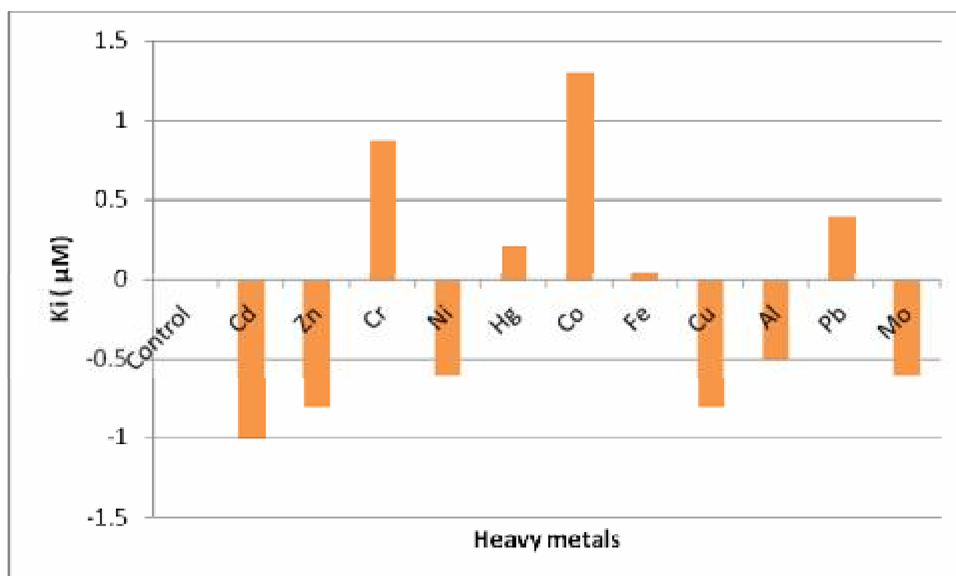


Figure (31)
K_i values for crude enzyme (POX) extracts of *Eruca Sativa* in control and in presence of different heavy metals at 400µM. Mean ±SD (n=3).

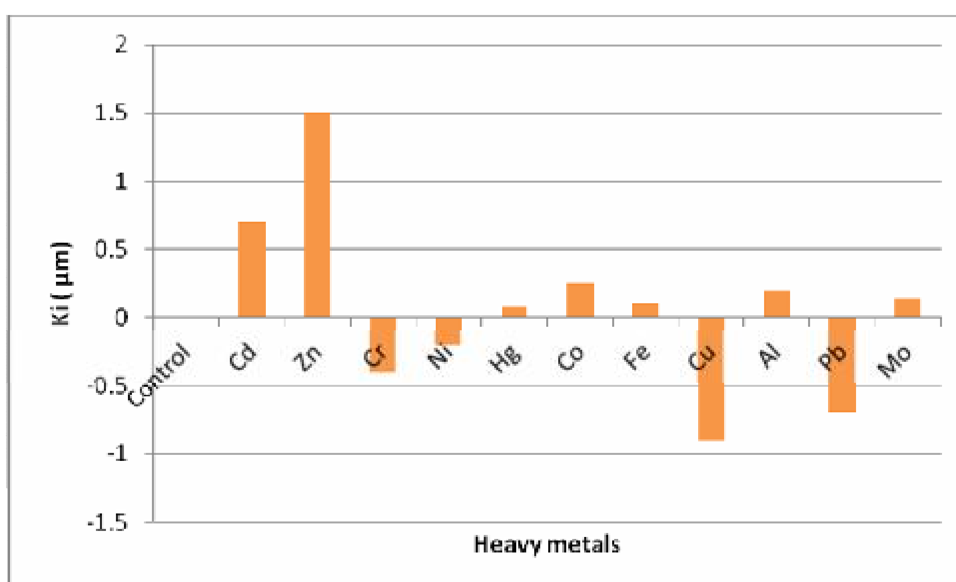


Figure (32)
K_i values for crude enzyme (PPO) extracts of *Rosmarinus oficinalis* in control and in presence of different heavy metals at 400µM. Mean ±SD (n=3).

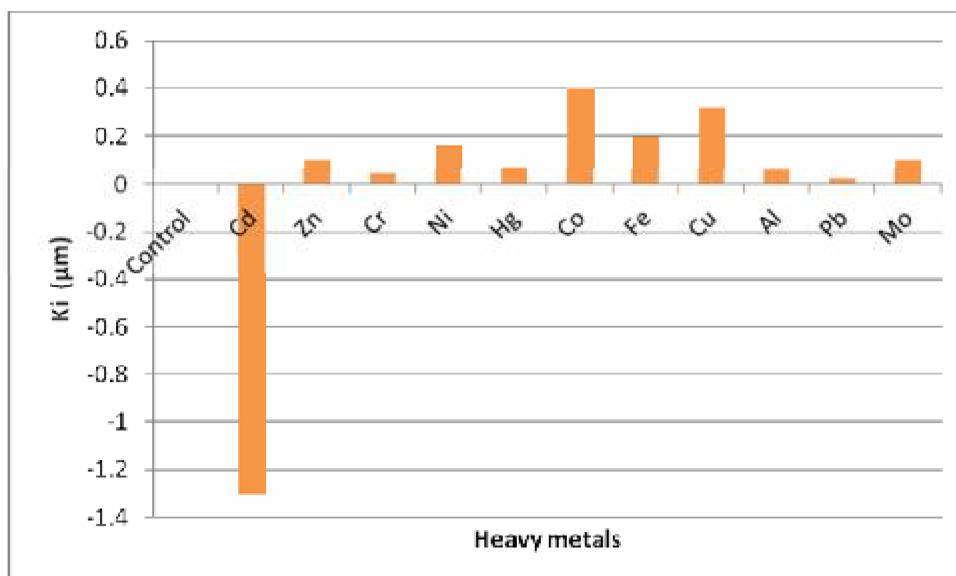


Figure (33)
K_i values for crude enzyme (PPO) extracts of *Petroselium cripsum* in control and in presence of different heavy metals at 400µM. Mean±SD (n=3).

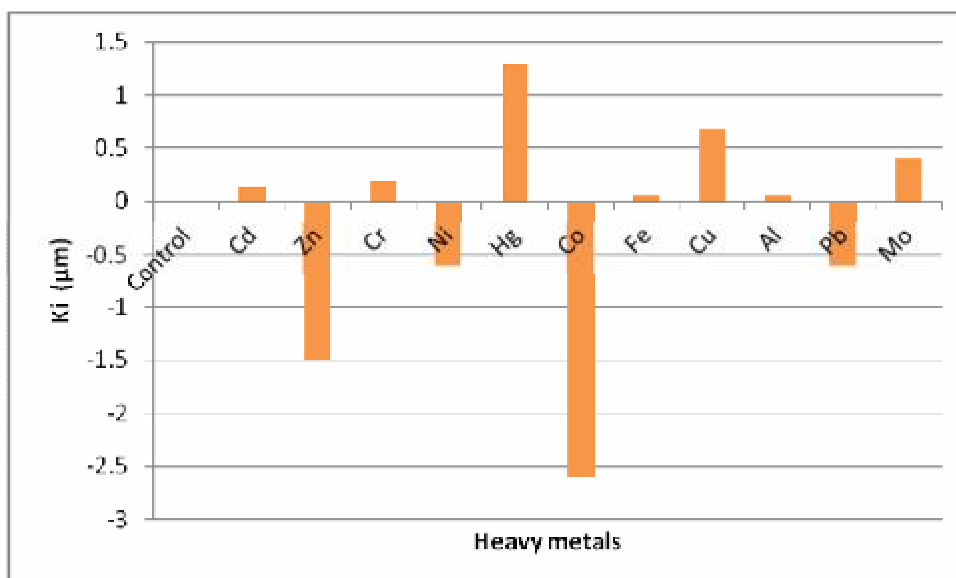


Figure (34)
K_i values for crude enzyme (PPO) extracts of *Eruca Sativa* in control and in presence of different heavy metals at 400µM. Mean ±SD (n=3).

السيرة الذاتية

الاسم: منار عبدالسلام البطوش

التخصص: ماجستير أحياء

الكلية: العلوم.

السنة: 2013م.